Project title	Sustaining UK fresh onion supply by improving consumer acceptability, quality and availability
Project number:	Fv 308
Project leader:	Mr David O'Connor, Allium & Brassica Centre Ltd
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Key staff:	Mr David O'Connor, Allium & Brassica Centre Ltd
Location of project:	
Project coordinator:	[Name, Organisation, Address]
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Date project completed (or expected completion date):	30 June 2010
Key words:	

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]
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Grower Summary

Headline

- Curing at lower temperatures had only marginal effects on the visual appearance of onion bulbs.
- Curing at lower temperature did not affect primary quality traits such as dry weight or pungency.
- To date there is no indication that the ethylene and 1-MCP treatments had any deleterious effects on bulb quality.
- Treatment, prior to curing, with ethylene or 1-MCP improved the efficiency of absorption.

Background and expected deliverables

Grower uptake and customer acceptance

- The bulb onion industry is highly co-ordinated and the uptake of new technology would be phased in over 2-3 years. Beneficial technology will be taken up universally as the industry now consists of mostly large professional enterprises.
- Improved consumer acceptability of product as it would be free from all pesticide residues at harvest.
- Improved maintenance of flavour and quality through better storage and shelf-life techniques.

Grower capital investment and cost recovery

- It is unlikely that significant investment will be needed as most onion producers have modern efficient storage facilities in place. Indeed, curing onions at lower temperature whilst maintaining quality will reduce costs.

Other benefits

- Reduction in emissions from direct firing of propane and electricity for fans and refrigeration. Saving *ca*. 180m MJ and 16,000 tonnes CO_2 p.a.
- Environmental benefit through avoidance of field spraying onions prior to harvest with the sprout suppressant maleic hydrazide (MH).
- Reduction of waste. Currently 17.5% of bulb onions are below retail quality and are used for processing. The target is a 30% reduction, leading to a 50% value

increase, saving £1.3 m p.a. A further 10% unsaleable waste is mostly composted / ploughed in. The target is a 30% reduction, with 3% more saleable = £1.5 m saving (total £2.8 m) p.a.

Science and Technology

Temporal and spatial profiling of identified biochemical and molecular markers will allow factors influencing dormancy / sprout suppression to be identified with possibilities to develop microarray techniques for screening cultivars and breeding for better storability and flavour. Knowledge of effects of endogenous ABA and ethylene in non-climacteric systems may be transferable to other non-climacteric UK fresh produce types.

Financial benefits

Commercial benefits

- Annual value in area of impact: 400,000 tonnes annual production with a farm gate value *ca*. £130 per tonne = £52 m p.a.
- Expected annual added value: Present drying / curing costs are £9.84 per tonne plus longer term refrigeration at £11.00 per tonne (energy cost only).
- 50% saving anticipated = £10.42 per tonne, with a total crop = £4.17 m gross margin improvement per annum.

Market potential

- Approximately 1 month additional supply 40,000 tonnes of UK onions = £5.2m addition farm gate sales per annum.
- Substitution of MH-treated imports 20% (90,000 tonnes imported from Holland in 2006). Hence 18,000 tonnes = £2.3 m additional farm gate sales per annum.

Action points for growers

- There are no recommended changes to current grower practice at this stage.

Milestones

Year 1

P1.1 Complete temporal and spatial biochemical analysis for onion bulbs held under different storage regimes used for 3.1 and 4.1 (CU; 18 months).

P2.1 Complete selection and addition of targeted genes for inclusion into onion oligonucleotide microarray and design (WHRI, 12 months).

P3.1 Complete evaluation of sprout suppressants/promoters (CU; 8 months).

P4.1 Select optimum curing regime to extend onion bulb storability and maintain quality and neck rot control (CU and ABC; 8 months).

Year 2

P1.2 Construct chemometric model for onion dormancy and sprout suppression (CU; 24 months).

P2.2 Complete design and fabrication of onion oligonucleotide microarray (WHRI; 24 months).

P1.3 Identify markers (chemometric and molecular) for dormancy induction, sprout suppression and attributes related to bulb quality (CU and WHRI; 24 months)

P3.2 Complete first ethylene and 1-MCP experiment (CU; 20 months).

P4.2 Establish the optimum curing regime with lowest energy input, which is still capable of providing sufficient control of postharvest neck rot (CU and ABC, 20 months).

Year 3

P2.3 Perform microarray hybridisations and data analysis on selected samples (WHRI, 34 months).

P3.3 Complete second ethylene and 1-MCP experiment (CU; 32 months).

P4.3 Complete optimisation of storage treatments in combination with energy usage reduction that prolong dormancy and inhibit sprout growth during storage (All; 36 months).

Science Section

Aim of Project

A more competitive and expanded UK onion industry based on energy efficient and residue-free long-term storage. Uptake of new guidelines ensured through HDC publications/events, BOPA Conference and on-farm workshops with established consultants within a well co-ordinated industry.

Introduction & Literature Review

Parts 1, 2 and 3.

Gemma Chope, Leon Terry – CU

Objective 1. Develop a chemometric model based on temporal and spatial profiles of biochemical markers linked to onion bulb dormancy induction and sprout suppression. (CU). [Years 1-2; Tasks T1.1 and T1.2]

Objective 4. Prolong storage and shelf life whilst reducing energy inputs by optimisation of postharvest treatments as monitored by biochemical and gene expression markers (All partners) [Year 1-3 Milestones T4.1, T4.2 and T4.3].

Part 2: Gemma Chope – CU, Andrew Thompson, John Hammond – WHRI

Objective 2. Produce the first microarray for onion to identify genes that are up- or down-regulated during dormancy and sprout suppression. (WHRI). [Years 1-2; Tasks T2.1, T2.2 and T2.3)

Part 3: Katherine Downes, Leon Terry – CU

Objective 3. Elucidate mechanisms related to dormancy and sprout suppression, as influenced by applied treatments, for maximum storage and shelf life (ABC, CU) [Years 1-3; Tasks T3.1, T3.2 and T3.3]

Profile the physiological and biochemical changes in long-term storage of onion bulbs when treated with ethylene or 1-MCP for 24 hrs before and after curing. The

objective is to understand the mechanisms by which ethylene and 1-MCP can be manipulates to achieve sprout suppression and whether pre- or post-curing application has any physiological or economical benefits

Onion bulb dormancy

Mature onion bulbs enter a dormant period, when sprouting and rooting are not induced despite favourable conditions. For most cultivars, true dormancy is relatively short, and ends early on in the storage period. Apparent dormancy is maintained through a period of sprout suppression when internal changes occur. These prepare the plant for subsequent growth and eventually the bulb proceeds towards flowering and seed production. Sprouting occurs when the leaf primordia that are produced in stored onion bulbs develop green leaves rather than scale leaves (Abdalla and Mann, 1963). The blades of these leaves elongate, and eventually protrude from the neck of the bulb. The growth rate of the sprout inside the bulb varies according to cultivar and pre- and postharvest factors such as maturity at harvest and storage regime. Sprout growth, and the suppression thereof, is a major factor in determining the storage life of onions.

Bulbs with roots sprout earlier in dry storage than those whose roots have been removed (Miedema, 1994b). Therefore, the root system may provide substances that promote sprout growth or elongation. Cultivar differences in time to sprouting in store are more pronounced in de-rooted bulbs than in rooted bulbs (Miedema, 1994b). Cytokinins produced in the roots stimulate cell division in the sprout meristem or increase the sink activity of the sprout. Wounding of the growth plate also promotes sprouting and may do so by facilitating gas exchange and promoting respiration.

Quality attributes of marketable onions

The aim of onion bulb storage is to meet consumer demands for extended availability of onions whilst maintaining product quality. The principal biological factors leading to onion bulb deterioration during storage are respiration, resumption of growth and pathogen attack.

Class I onions must not show any signs of external sprouting (Commission Regulation 1508/2001/EEC). Early signs of external shoot growth are permitted in Class II onions provided that the number or weight does not exceed 10% per unit of

presentation. Bulbs with watery scale and bacterial or fungal rots are deemed unfit for marketing.

Strategies to delay sprouting

Storage life of onions depends on many factors such as cultivar and pre- and postharvest treatment. Long-storing cultivars are available and are characterised by high dry matter content. Research to develop strategies to delay sprouting has been focused on crop husbandry, the characteristics of the storage environment and breeding programmes.

Pre-harvest treatment and conditions in the field have an important role to play in affecting storage life. These include pre-harvest nutrition, temperature during the growing season, application of maleic hydrazide, crop maturity at harvest and the harvesting process.

Postharvest treatments and storage conditions have a significant impact on storage life. These include curing and drying, irradiation, nitrous oxide treatment, and aspects of the storage environment including temperature, gaseous composition of the atmosphere, and humidity.

Maleic hydrazide

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione; MH) is a chemical isomer of uracil that is applied as a pre-harvest spray to inhibit subsequent sprouting of bulbs in store (Figure 1.1.1).

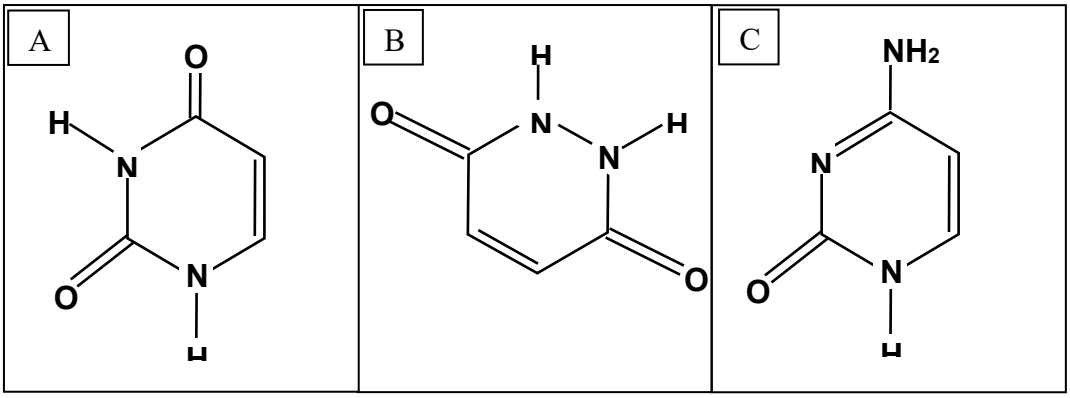


Figure 1.1.1. Chemical structure of: A – Uracil, B – Maleic hydrazide, C – Cytosine.

Responses to MH vary with cultivar (Sorensen and Grevson, 2001). Timing of application as well as dose is important when considering the residue level in the crop. A ban on the use of MH in Denmark is being considered because of the risk of it leaching into drinking water (Sorensen and Grevsen, 2001) and concerns over its mutagenic properties (Marcano *et al*., 2004). The amount of MH used in onion production in Denmark was reduced by 75% in a three-year period from 1997 (Sorensen and Grevson, 2001).

MH is incorporated into the RNA of cells where it is substituted for cytosine (Benkeblia, 2004). In normal root tip cells of A. cepa the ultrastructure of the nucleolus has mixed granular and fibrillar components. However in bulbs treated with MH, the granular components were centrally located and surrounded by the fibrillar components, an arrangement known as nucleolar segregation (Marcano et al., 2004). This is a morphological manifestation of blocked transcription. In this way MH affects the biosynthetic activity of the nucleolus. Maleic hydrazide caused a dose dependent reduction in the mitotic index (the mitotic index allows estimation of the frequency of cell division) compared to untreated bulbs whereby the mitotic index remains constant. The effect of MH concentration was 1.93-fold higher than the effect of the time of exposure. Maleic hydrazide was also capable of breaking chromosomes. However, MH had no effect on sugar and organic acid composition of onion bulbs cv. Sentinel (Salama et al., 1990).

Crop maturity at harvest

The developmental stage of the crop at harvest impacts on both yield and storage potential. The consensus in Europe and the USA is that the optimum harvest time for storage onions is at 80-90% tops down, sacrificing some yield for a greater number of intact skins (Gubb and MacTavish, 2002). If bulbs are harvested too soon the water content in foliage leaves and the neck is too high, which results in increased susceptibility to pathogen attack. Early harvested bulbs may not be dormant and would therefore be unsuitable for storage purposes. Maturity stage at harvest can influence initial bulb weight, respiration and incidence of sprouting, decay and cumulative weight loss. Rutherford and Whittle (1982) found that bulbs harvested early, dried and stored in the same manner as bulbs harvested later, yet had lower carbohydrate levels, which were further reduced during sprouting, which occurred earlier.

Curing and drying

UK Onions for storage are cured and dried after harvest (O'Connor, 1979; Gubb and MacTavish, 2002). It is important that the skin integrity, firmness, colour and flavour are maintained during curing. The purpose of curing is to dry the thin outer layers of the bulb to form one or more complete outer skins. These outer skins act as a barrier against water loss and infection from fungal pathogens such as Botrytis allii (neck rot) (Maude et al., 1984), Aspergillus niger (black mould) and Fusarium oxysporum (basal rot), and bacterial pathogens such as Erwinia carotovora (soft rot) (Fenwick and Hanley, 1985). Curing is complete when the necks have dried out and are tightly closed, and the skins have an attractive colour (O'Connor, 1979). The time this takes depends on the temperature and relative humidity of the forced ventilating air and the maturation stage of the bulbs. Standard practice is to dry the bulbs in bulk stores using air at 30ºC. After three to five days the temperature is lowered to 24ºC and relative humidity (RH) to 70-75% to complete the curing process. The crop is then slowly cooled to the desired storage temperature.

Storage environment

Temperature, humidity and gaseous atmosphere can be manipulated to increase the storage life of onion bulbs. The most important of these is temperature. The storage regime chosen depends on the cultivar, target storage period and cost.

Temperature has a profound effect on the dormancy period and storage life of onion bulbs. In general, sprouting is inhibited both by low and by high temperatures, and encouraged at intermediate temperatures (Abdalla and Mann, 1963; Brewster, 1977; Miedema, 1994a; Ernst *et al*., 1999). Different cultivars respond differentially to temperature (Gubb and MacTavish, 2002). The optimum temperature range for sprouting in dry storage is 10-20 ºC for most cultivars, with some cultivars displaying a sharp optimum while others have a broader range. Moisture loss is greater at temperature ranges <10 ºC and >27 ºC.

In developed temperate countries, such as the UK, onions are kept in large, specialised stores. Ventilation is forced, and temperature is usually maintained around 5 ºC, but can be as low as -1 ºC. In warm climates, such as the tropics, high temperature storage is a practical option, but involves a compromise between sprouting losses and rotting losses (Ko *et al*., 2002). High temperature storage conditions are generally 25-30 ºC and 60–75% RH. Ventilation of storage bins to reduce fluctuations in temperature and humidity reduced the rate of external sprouting, bacterial infection and dehydration over 31 weeks of high temperature

storage in red onion cv. Baftain bulbs (Brice *et al*., 1995). The high temperature inhibition of sprouting may be related to the dormancy observed in hot seasons in some wild *Alliums* (Gubb and MacTavish, 2002). Short-term (three weeks) high temperature postharvest treatments of 30 and 35 ºC significantly reduced the number of days to sprouting in dry storage at 15 ºC, when compared to those exposed to postharvest temperature treatments of 15 and 25 ºC, which in turn were not significantly different from one another (Miedema, 1994a). This indicates that exposure of onion bulbs to high temperatures during curing and drying may reduce the level of dormancy and therefore reduce storage time.

Short-term (two or three weeks) chilling treatments at 0 or 9 °C decreased the time to sprouting in onion cv. Rouge Amposta bulbs subsequently stored at 18 °C, with the 9 °C treatment for three weeks having the greatest effect – 100% of bulbs in this treatment group had sprouted after 4-5 weeks, but after 8 weeks only 20% of non-chilled bulbs had sprouted (Benkeblia and Selselet-Attou, 1999a). The chilled bulbs also generally had a lower concentration of soluble sugars. Therefore, it is important that the chilling treatment is maintained long-term in order to extend storage life. Short-term chilling treatment may mimic the conditions that a dormant onion would experience over winter, with the return to a higher temperature being equivalent to the onset of spring and a trigger for release from dormancy.

Controlled atmosphere (CA) storage involves manipulating the oxygen and carbon dioxide concentrations in the storage environment in addition to the temperature (Gubb and MacTavish, 2002). Low oxygen storage inhibits sprouting, decreases the incidence of neck rot and reduces weight loss. However, very low oxygen concentrations (0.7%) can cause high rates of sprouting after removal from storage, as well as off-odours and tissue breakdown. Also, high carbon dioxide concentrations (>10%) for short-term storage can cause accelerated softening, rooting and a putrid odour. A storage atmosphere consisting of 5% CO_2 and 3% O_2 has been found to yield a good percentage of marketable bulbs and maintain quality (Adamicki and Kepka, 1974; Smittle, 1988).

The relative humidity of the storage environment is a compromise between maintaining a level below that at which pathogens are encouraged and above that at which water is rapidly lost from the bulbs (Hole *et al*., 2000). The outer skins that protect against water loss tend to crack and fall off at <55% RH, and pathogen attack

is encouraged at >80% RH, therefore 55-80% RH is desirable in the storage environment. When the water content of the skin is in equilibrium with the water vapour pressure of the surrounding atmosphere, water will be adsorbed or desorbed depending on the relative pressure. Changes in humidity, therefore, have an impact on the properties of onion skins. This is significant as the ability of onion bulbs to withstand physical abuse during postharvest and post storage handling depends on the mechanical properties of the skins. Hole *et al.* (2000) found that humidification increased the resistance of skins to breaking. Compliant skins are better able to withstand the rigours of commercial handling. Manipulation of humidity to condition skins reversibly prior to post storage handling may have a positive impact on bulb quality.

Alternative strategies to delay sprouting

Increasing pressure from both consumers and retailers to provide food with little or no chemical residues means that the continuing use of MH to extend onion storage life is far from certain, and alternative strategies must be explored. In order to identify potential targets for manipulation to suppress sprouting in store, it is useful to examine what changes occur in stored onion bulbs. Many characteristics change during storage including water content, and the concentration of flavour compounds, carbohydrates, minerals and plant growth regulators. Changes in these characteristics are likely to be linked with respiration and remobilisation of carbohydrates to provide energy for the growing sprout. All nutrients required for growth of the sprout must come from within the bulb; therefore changes in certain key characteristics could be used to predict the onset of sprouting. Peaks and troughs in certain substances are known to coincide with sprouting but there is currently no biochemical assay that anticipates sprouting.

Plant growth regulators

During over winter storage in the UK a gradual change in the relative composition of plant growth regulators occurs as the levels of growth inhibitors drop and the levels of growth promoters rise. Hormone activity in onions cvs. Rijnsberger (long-storing) and Lancastrian (short-storing) bulbs was measured by Thomas (1969) and Thomas and Isenberg (1972). The following pattern existed (Figure 1.1.2); gibberellins (GAs) had a first peak in December, followed by peaks of cytokinins and auxins. High auxin activity persisted as sprouting continued. A second GA peak was accompanied by sprouting in March. This GA peak was more likely to be an effect of sprouting rather

than a cause, as GA activity was low in both non-sprouted and internally sprouted bulbs (Thomas, 1969; Yamazaki *et al*., 2002) and application of exogenous GAs and auxin failed to stimulate sprouting (Thomas, 1969).

Abscisic acid (ABA) has been identified as an inhibitory substance in onion bulbs (Tsukamoto *et al*., 1969). The concentrations of inhibitors in bulbs with internal signs of sprouting were low when compared with the levels in non-sprouting or fully sprouted bulbs. More inhibitor was present in the long-storing cultivar at the beginning of the storage period than in the short-storing cultivar (Thomas, 1969). Therefore, before an external sprout is visible, important internal changes occur. The roles and mode of action of plant growth regulators are unknown, but it is probably a complex phenomenon involving the combined action of several endogenous hormones (Gubb and MacTavish, 2002).

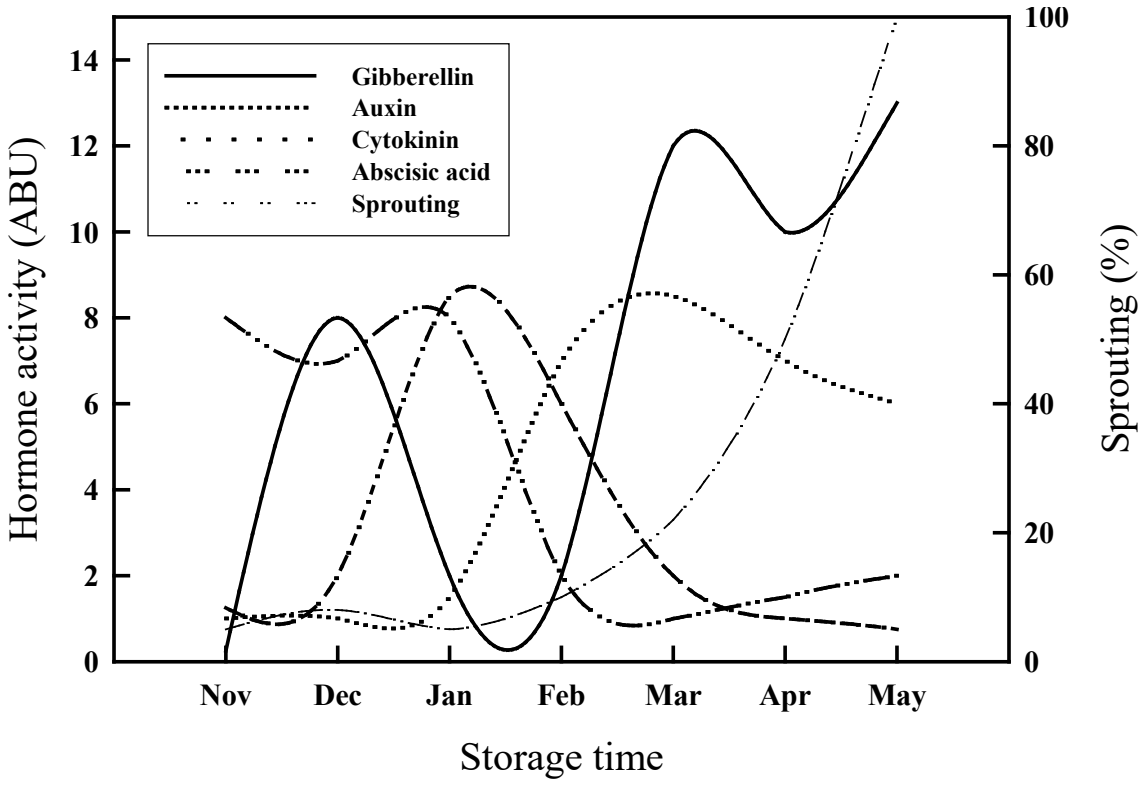


Figure 1.1.2. Percentage sprouting and hormone activity of onions cv. Rijnsberger stored at 5-8°C (Thomas and Isenberg, 1972).

Continuous exposure of onion bulbs to ethylene in store has recently been put forward as a method of sprout suppression (Johnson, 2006). However, there are conflicting reports on the effect of ethylene during storage. Abdel-Rahman and

Isenberg (1974) observed that onion cv. Elba Globe bulbs produced ethylene at much greater concentrations at the end of dormancy than at the beginning. In contrast, Benkeblia and Selselet-Attou (1999b) found little variation in the ethylene production by onions cv. Rouge Amposta. Ethephon (2-chloroethylphosphonic acid, CEPA) degrades to form ethylene in an alkaline solution (Yang, 1969). Application of ethephon to plants has been effective in causing responses characteristic of ethylene treatment (Benkeblia and Selselet-Attou, 1999b). A combination of cold storage (9 °C for three weeks) and the injection of 1 ml of a 100 mg l^{-1} solution of ethephon into the centre of the bulb caused onion cv. Rouge Amposta bulbs stored at 18 °C to sprout earlier (50% sprouting after 2 months, 100% after 4 months) than those treated with ethephon alone and untreated controls (50% sprouting after 3 months, 100% after 6 months). It is likely that it was the effect of the chilling treatment that reduced the storage life of the bulbs; however, as no bulbs were subjected to chilling alone, this cannot be proven. Injection of bulbs with ethephon alone had no effect on sprouting, but when applied in combination with exogenous ABA it reduced the effect of ABA on the dormant period (Abdel-Rahman and Isenberg, 1974).

Abscisic acid is a naturally occurring phytohormone. The ABA biosynthesis pathway (Figure 1.1.3) begins in chloroplasts and other plastids with the cleavage of a C_{40} carotenoid precursor to form xanthoxin. In the cytoplasm, xanthoxin is converted to ABA via abscisic alcohol (Cutler and Krochko, 1999; Taylor *et al*., 2005). ABA has many physiological effects, many related to the response to water and cold-stress, including bulb and seed dormancy, inhibition of germination, stomatal closure and inhibition of cell elongation. The amount of ABA in the plant is a balance between synthesis and degradation. Plant development, environmental conditions such as drought stress, and other growth regulators affect these processes.

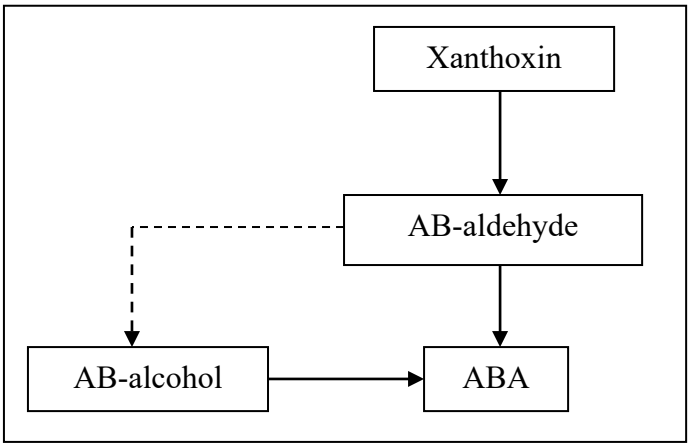


Figure 1.1.3. The synthesis of abscisic acid (ABA) from xanthoxin in higher plants. Solid arrows indicate the major pathway, dashed arrows indicate a minor pathway, AB=abscisic (Cutler and Krochko, 1999).

The predominant pathway for ABA metabolism is hydroxylation at the 8' position, catalysed by the enzyme abscisic acid 8'-hydroxylase, to give 8'-hydroxy-ABA which is unstable and readily converts to phaseic acid (PA). In turn, PA can be reduced to dihydrophaseic acid (Cutler and Krochko, 1999; Nambara and Marion-Poll, 2005) (Figure 1.1.4). Phaseic acid has little hormonal activity in most assays and can be isolated, whereas 8'-hydroxy-ABA still has some hormonal action but can not be easily isolated due to its instability.

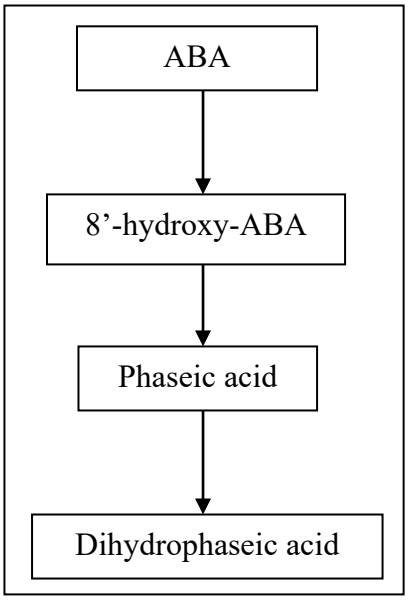


Figure 1.1.4. Metabolism of abscisic acid (ABA).

Endogenous ABA is found in all onion tissues *viz*. leaf, growing tip, bulb and leaf sheath (Matsubara and Kimura, 1991), and accumulates in all tissues during the growing period. The actual concentration of ABA varies according to plant age and tissue type.

The endogenous ABA concentration is affected by tissue water status and is high in water-stressed plants. During bulb formation storage molecules accumulate, which facilitates changes in tissue water status. *Allium wakegi* (a cross between Japanese bunching onion and shallot) plants do not form bulbs under short day conditions, and the endogenous ABA concentration remained low when plants were subjected to short days - a maximum concentration of 5 ng g^{-1} FW compared to a maximum

concentration of 20 ng g^{-1} FW in plants subjected to long day conditions was observed (Yamazaki *et al*., 1999a). The ABA concentration increased during bulb development, reaching a maximum two weeks after harvest: a rise from 1 ng g^{-1} FW to 13 ng g^{-1} FW over two months. This change was associated with changes in water status during bulb formation (Yamazaki *et al*., 2002). However, *in vitro* studies do not support the theory that ABA is the primary cause of bulbing; addition of exogenous ABA (0.1-5 mg l^{-1}) to *in vitro* cultured onion plants did not induce bulbing, although the number of leaves decreased (Matsubara and Kimura, 1991). In addition, fluridone (an inhibitor of ABA biosynthesis) treatment of *A. wakegi* plants reduced endogenous ABA concentration, but did not inhibit bulb scale formation or affect leaf sheaf ratio (ratio of leaf sheath length to the length the oldest unexpanded leaf) relative to untreated control plants (Yamazaki *et al*., 1999a).

Abscisic acid has been associated with dormancy in onions and other plants with storage organs such as potato (Suttle and Hultstrand, 1994; Destefano-Beltrán *et al*., 2006a; Destefano-Beltrán *et al*., 2006b). Current understanding is that ABA is synthesised in the leaves and translocated to the bulb throughout growth. Treatments to prematurely kill off the aerial parts of onion plants resulted in increased sprouting of bulbs in store (Thomas and Isenberg, 1972), perhaps due to a reduction in accumulation of ABA in the bulb because ABA cannot be translocated from dead leaves. Bulb ABA concentration reached a maximum shortly after harvest (Yamazaki *et al*., 2002). Onions are harvested when the aerial parts of the plant have fallen and would be expected to be less metabolically active. This may suggest that ABA can be synthesised in other parts of the onion plant when the aerial parts have been removed or ceased to be metabolically active.

The current situation

Long-term storability of onions is a result of first, dormancy induction and then sprout suppression (section 1.1.1), and little attention has been given to understanding the underlying physiological and genetic mechanisms. The UK has led the world in many aspects of bulb onion drying and storage technology, which was based on 1970's research at Kirton EHS and MAFF Development Farm Project (section 1.1.3.3). In light of rising energy costs (the cost of commercial electricity increased by *ca*. 50%, and gas by *ca*. 65% between 2005 and 2006; Department of Trade and Industry, 2006) any reduction in the amount of gas and electricity used in the curing, drying and storage of onions would be desirable to industry. Current UK practice aims to remove surface moisture within three days of loading the store by heating at

30 ºC, followed by a further ten days at 24ºC (RH not to exceed 75%). The crop is then allowed to cool to approximately 15 ºC, with ventilation, for a few days until the necks are tight and dry. The temperature can then be slowly reduced until the desired long-term storage temperature is reached. The curing and drying procedure is based on a method developed in the 1970s (Shipway, 1977; O'Connor and Shipway, 1978), and therefore the research that formed the basis of this procedure was carried out on cultivars that are very different from those used today. Thus, it is likely that alterations to current methods, such as a reduction in the temperature and duration of the curing and drying periods could deliver benefits in the form of energy savings and reduced carbon emissions, while still producing onion bulbs of a satisfactory quality standard.

Since that time, onion growers in the UK have invested heavily in technologically advanced refrigerated and controlled atmosphere (CA) storage systems designed to maintain availability of UK onions for prolonged periods. The current annual production of around 400,000 tonnes (value £52m) meets around two thirds of the UK annual requirement for onions. The use of refrigerated and CA storage systems is effective but is also energy intensive, depends upon the use of the sprout suppressant, MH. In the current climate of ever-increasing energy costs (up by 50% in 3 years), the viability of these energy intensive methods of UK onion production and storage are being threatened. There are also increasing pressures to from both consumers and retailers to eliminate residues in food, which makes the future use of MH as a sprout suppressant uncertain. The UK system of topping onions in the field, lifting into store and then drying at 28ºC may not be so well suited to earlier maturing modern cultivars and may be adversely affecting storage life.

Recent work (Chope *et al*., 2006; 2007a) has shown for the first time that a decline in endogenous abscisic acid (ABA) was correlated with storage life under both controlled atmosphere conditions (Figure 1.1.5) and regular atmosphere conditions (Figure 1.1.6). It was also shown that the concentration of ABA in freshly harvested bulbs was approximately double that measured before versus after curing (Chope *et al*., 2007b). Extended curing times may also reduce ABA concentration. Yamazaki *et al*. (1999a; b) also demonstrated a functional role of ABA in maintaining bulb dormancy in *A. wakegi*. Thus, maintaining ABA concentration could extend dormancy and storage life.

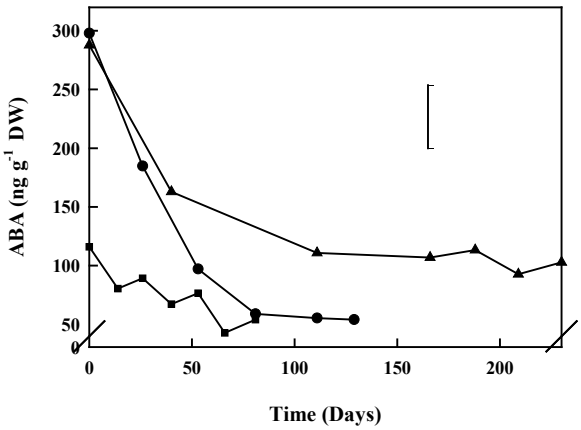


Figure 1.1.5. Changes in the abscisic acid concentration in bulbs of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) during controlled atmosphere storage. LSD bars (P=0.05) are shown.

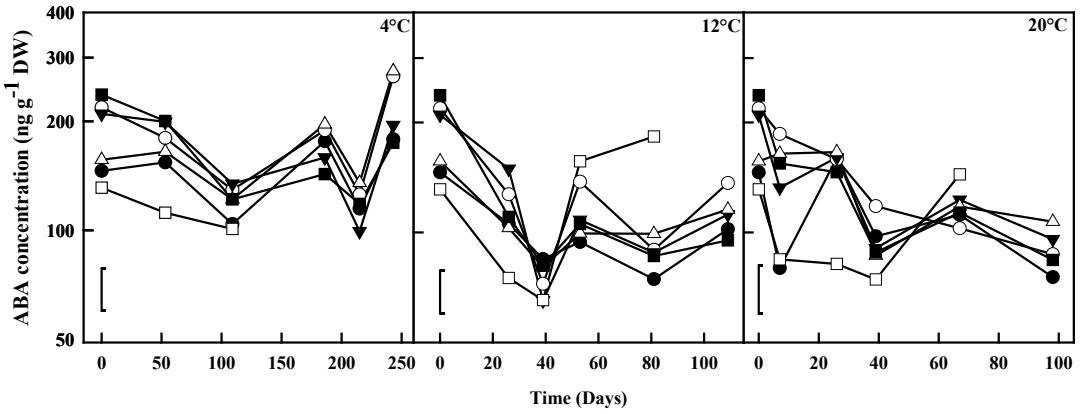


Figure 1.1.6. The change in mean abscisic acid (ABA) concentration in bulbs of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (Δ), Renate (■) and SS1 (□) stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=20). LSD bars (P=0.05) are shown.

Onions are regarded as non-climacteric in their response to ethylene, and, as such, have consistently low endogenous ethylene levels and lack the dramatic change in ethylene production found in climacteric fruit which triggers ripening (Giovannoni, 2001). However, both the continuous application of ethylene gas and the pre-harvest application of Ethephon have both been shown to increase the storage life of onions (Adamicki, 2005; Johnson, 2006). Ethephon is an ethylene yielding chemical which when applied to plants can elicit a response characteristic of ethylene treatment

(Yang, 1969; Warner and Leopold, 1969). Application of Ethephon two weeks prior to onion harvest has been found to prevent sprouting during storage however there was no significant effect on rooting (Adamicki, 2005). In 2003, the Restrain Company Ltd. was established in the UK producing a system which converts ethanol into ethylene and water using a catalyst. The system is designed to generate ethylene throughout onion and potato storage and has shown reductions of internal shoot growth of up to 70%. Unlike Ethephon treatment, Restrain has been found to increase shelf-life after 5.5 months in cold storage for 14 days at 20°C (Adamicki, 2005; Johnson, 2006). Benefits of ethylene treatment include the elimination of detectable residues, the use of low concentrations (10 µl l^{-1}) which pose no hazards to workers and the potential integration of ethylene treatment with controlled atmosphere, high and low temperature storage (Johnson, 2006).

1-methylcyclopropene (1-MCP) is an ethylene perception inhibitor that is thought to bind to ethylene receptors preventing ethylene binding and eliciting a response (Blankenship and Dole, 2003). It has been suggested that 1-MCP has 10 times the affinity for ethylene receptors than ethylene itself and that 1-MCP is active at much lower concentrations (Sisler and Serek, 1997). Further research at Cranfield University demonstrated that the storage life of mild onions cv. SS1 could be increased by applying the ethylene binding inhibitor, 1-methylcyclopropene (1-MCP), before storage (Chope *et al*., 2007c). Application of 1-MCP also retained bulb firmness and maintained a 2-fold higher concentration of glucose and fructose after 50 days storage at 12 ºC as compared to control (Figure 1.1.7). The effects of 1-MCP did not appear to be mediated by ABA.

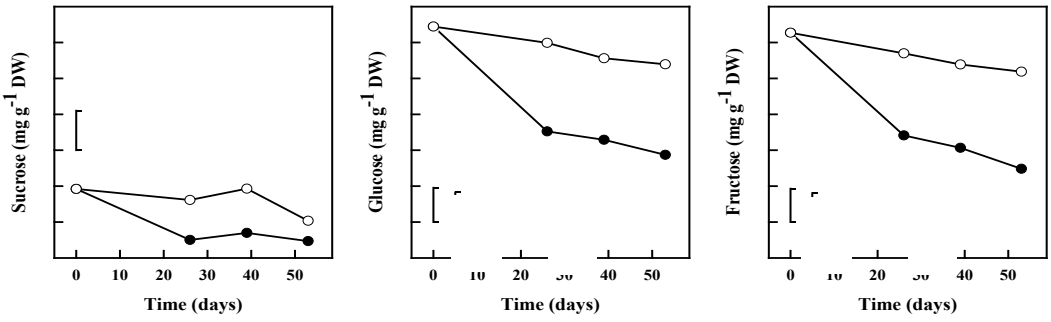


Figure 1.1.7. Sucrose, glucose and fructose concentrations in onions treated with 1 µl l^{-1} 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 12ºC for 53 days or 20ºC for 39 days. LSD bars (P=0.05) are shown.

This dichotomy demonstrates that both ABA and ethylene play a vital role in onion dormancy and sprout suppression, and might be manipulated to extend storage and shelf-life. Different onion cultivars vary radically in their innate dormancy and storage, but relatively little is known of the fundamental basis of this variation other than largely empirical correlations to such traits as dry matter, pungency, skin quality and degree of polymerisation of non-structural carbohydrates. There is an opportunity, with this proposal to maintain and enhance the UK technological lead in temperate onion production with a total revision of storage practice, significant improvement in energy efficiency during curing, drying and storage and to discontinue use of MH sprout suppressant, whilst not compromising on product quality As with any new treatment it is important to consider any potential negative influences on produce quality. In particular, the antioxidant capacity of onion bulbs is produced from vitamins, flavonoids, phenolics and sulphur compounds. Onions are one of the major sources of dietary flavonoids of which the major flavonoid is quercetin. Quercetin is found in conjugated forms as quercetin 4'-*O*-*β*-glucopyranocide, quercetin 3,4'-*O*-*β*-diglucopyranocide and quercetin 3,7,4'-*O*-*β*-triglucopyranocide (Sellappan and Akoh, 2002), these compounds are effective against non enzymatic lipid peroxidation and oxidation of low density lipoproteins (LDLs) (Corzo-Marínez *et al*., 2007). Phenolics are secondary metabolites characterised by hydroxylated aromatic rings and are mainly formed in onions from anthocyanins and flavonoids (Benkeblia, 2000). Phenolics and flavonoids have been found to contribute to the antioxidant properties of onions with positive correlations with radical scavenging activity and antioxidant activity (Nuutila *et al*., 2003).

Genomes within the *Alliums* are large (King *et al*., 1998). Onions are diploid (2n=16), with an estimated nuclear genome size of 15 290-15 797 Mbp per 1C, and a 2C DNA amount of 31.69 - 33.2 pg (Arumuganathan and Earle, 1991; Ricroch and Brown, 1997). This is 6, 16 and 107 times larger than maize, tomato and *Arabidopsis,* respectively (King *et al*., 1998). The guanine-cytosine (GC) content is a characteristic of a genome which is sometimes used to classify organisms taxonomically. Genes tend to have a higher GC content than the rest of the genome. The GC content of the *A. cepa* genome is *ca*. 37% (Kirk *et al*., 1970, Ricroch and Brown, 1997). There is a paucity of public genetic information on onion or any other *Allium* crops. Some key genes in the sulphur assimilation pathway have been cloned (McCallum *et al*., 2002) and recently, a set of over 10 000 onion expressed sequence tags has become available (Kuhl *et al*., 2004). However, biochemical and molecular

investigation are required before the exact function of these genes and the proteins they encode can be assigned (Jones *et al*., 2004).

Some genetic studies have already been carried out in onions. A low density genetic map has been produced consisting of 116 markers on 12 linkage groups and covering 1064 cM, with an average distance of 9.2 cM between loci (King *et al*., 1998). Significant positive, genetic and phenotypic correlations have been found among total soluble solids, dry matter, pungency and onion induced anti-platelet activity (Galmarini *et al*., 2001). More recently, the map has been expanded to include an extra 10 markers and now spans more 1907 cM and all eight chromosomes (Martin *et al*., 2005). This genetic map has been used with quantitative trait loci (QTL) analysis to identify a locus (*Frc*) that affects bulb fructan content to chromosome 8 (McCallum *et al*., 2006).

Advances in the field of molecular biology have meant that higher throughput techniques, such as microarrays are available. It would be beneficial to assess molecular markers of sprout suppression and dormancy in conjunction with physical and biochemical traits, as this will further elucidate the genetic mechanisms underlying these physiological processes. Microarray technology would allow simultaneous screening of thousands of onion genes. The expression of these genes could then be compared with desirable bulb traits. In addition, this technique would reveal classes of genes whose expression alters during different environmental, temporal or spatial conditions.

There are two possible approaches to producing a microarray chip suitable for this use. Highly detailed genetic information on model plants is available in public databases. Nevertheless, there is a limit to the validity of the application of data from model plants to crop species (Havey, 2004). Genetic resources for other important crop species, such as rice, are also available, however there are important differences between the *Poales* to which rice belongs, and the *Asparagales*, to which onion belongs (Kuhl *et al*., 2004) which mean that a microarray constructed using sequences from rice would be unlikely to be a total success. The second option would be to construct an onion-specific microarray. There are 11,726 unique sequences available on the TIGR database (The Institute for Genomic Research, 2003; Table 1.1.1), of which 3838 have tentative consensus (TC) status, and have been allocated unique 70-mer oligonucleotide sequence suitable for construction of microarrays (Richmond and Somerville, 2000).

Table 1.1.1 Origin of expressed sequence tags from onion.

TIGR Library Code	No. of ESTs	Tissue
#ORD	84	Seedling leaf
#ORE	106	Not clear
#OSA	14	Re-sprouted bulb
#9A9	477	Sulphur deprived roots
#ALI	483	Bulb meristem
T11531	18389	Mixed callus, young bulb and root

This collection of 3838 oligonucleotides could be expanded by mining genomic databases, and adding specific genes related to dormancy such as ABA and ethylene metabolism and signaling, and carbohydrate metabolism. In cases where these target genes could not be located on public databases, genes identified by RT-PCR using degenerate primers designed from sequences aligned from other monocotyledonous species could be added. Microarray analysis should be accompanied by detailed metabolomic profiling, as it is likely that post-transcriptional regulation of proteins occurs.

A complex chemical profile including substances such as ABA and ABA metabolites, compounds concerned with sulphur and carbohydrate metabolism as well as the parameters measured in the current study (pyruvate, fructan, fructose, glucose, sucrose, TSS) would allow the application of chemometrics to the gene expression data. Chemometrics is a statistical technique whereby the chemical data collected can be related to the physiological state (Prazen, 2005). In this way it is anticipated that the use of metabolomic profiling in conjunction with microarray technology could identify the factors that are important in the transition from dormancy induction to sprout suppression. The onset of sprouting occurs at different times in different cultivars, and there is also considerable variation within cultivars. In potatoes this issue was overcome with the use of bromoethane (BE) which chemically induced premature breaking of dormancy (Destefano-Beltrán *et al*., 2006a). If a similar treatment could be applied to onions then this would be advantageous as it would be possible to control when the break of dormancy occurred. However, consideration would have to be made as to whether the chemically-induced dormancy break occurred via the same mechanisms as the natural process.

Potentially, diagnostic microarray chips based on a reduced number of specific sets of genes that were related to specific physiological stages and/or quality traits could be developed. Mass production of these slides would reduce the costs involved. Diagnostic tests that could predict sprouting would be beneficial for growers to predict both the storage life and the shelf life of the crop. The chip could also be used as a replacement in the future for time consuming full biochemical and physical profiling, but microarray analysis would need to be carried out in the laboratory. If sets of genes that were differentially expressed on sprouting were identified then transcription factors that control the initial stages of meristematic activity could be targets for genetic modification. However, this approach may be problematic, because if re-growth was delayed indefinitely then seeds or sets could not be produced from the modified bulbs. Also there is still considerable consumer hostility towards genetically modified crops.

Materials and methods

Part 1: Gemma Chope, Leon Terry – CU

Plant material

Three onion cultivars with varying storage potential were selected *viz.* Red Baron (average-storing), Wellington (long-storing) and Sherpa (average-storing) and grown to normal commercial practice on 3 x 0.75 acre sites: Elveden Farms, Nr. Thetford Norfolk (sand); A. Findlay's, Cardington, Beds (sandy clay loam); G's nr Littleport, Cambs (peat). The intention was to harvest only from two sites, holding one in reserve in case of crop failure. The two sites selected for harvesting were Elveden and Findlay's. The site at Elveden was drilled 9th March 2007 at a rate of 47-48 plants m^{-2} and bulbs were hand-harvested at 100% fall-over into bins on 2nd September. The site at Findlay's was drilled on 28th February 2007 at a rate of 37-38 plants m^{-2} and were machine-harvested at 80% fall-over on 16th September. See Appendix for crop diaries.

Fields trials – July 6

Elveden

Findlay

Postharvest treatments and storage

The following postharvest curing treatments were applied, beginning 5th September 2007 for onions from the Elveden site, and on 19th September 2007 for onions from the Findlay's site. Experimental bulbs were placed in nets among loose bulbs in 1 tonne wooden boxes.

- 28ºC for six weeks. RH 65-75%.
- 24ºC for six weeks. RH 65-75%.
- 20ºC for six weeks. RH 65-75%.

Data on the temperature, heating hours, humidity and humidity and humidifier hours for the curing rooms are presented in the Appendix. Following the postharvest curing treatments the nets were removed from the boxes, and the experimental onions were placed into plastic crates and stored at 0 ± 1 ºC at Cranfield University. In addition, onions from the bulk boxes were transported to the Allium & Brassica Centre for commercial storage and evaluation of sprouting and deterioration to take place around the end of May 2008.

Bulk storage at ABC

24ºC Curing **20ºC Curing**

Sampling strategy and experimental design

The experimental design was a completely randomised design with three replicates. At both sites the plot was divided into three sections with onions harvested from each of the three sections being kept separate and treated as replicate blocks to be taken from the field to the store. Samples were taken straight after harvest (day 0), after 6 weeks postharvest curing treatment and at four regular intervals during cold storage. Five bulbs were sampled for each replicate and treatment combination, making a total of 1620 bulbs for the entire experiment. Each bulb was cut each onion in half from top to bottom. One half of each bulb was used to provide tissue for pungency analysis. From the remaining half, two eighths were cut, wrapped separately in foil and immediately snap frozen in liquid nitrogen. One set of samples were stored at -40ºC prior to lyophilisation, and the other stored at -80ºC for use as a source of RNA.

Physical measurements

Sprout length

The bulb was assessed for the presence of a sprout after being cut in half. If a sprout was present, the length of the sprout, and the length and height of the bulb was recorded, along with the colour of the sprout i.e. white, pale yellow, yellow, pale green, green.

Colour

All loose and/or incomplete outer skins and roots were removed. Colour was assessed using a Minolta, and was expressed in terms of lightness (L*), chroma (C), and hue angle (Hº). Each was the mean of three measurements taken from around the equator of the onion. Following colour assessment, the tough outer skins were removed and a sample of outer skin equivalent to that covering half a bulb was retained.

Biochemical measurements

Pungency

Pyruvate concentration was measured as per final report for HL0164.

2.1.5.2 Extraction and quantification of non-structural carbohydrates

Carbohydrates in onion bulbs were extracted and quantified according to Davis *et al.* (2007) and that detailed in final report HL0164.

2.1.5.3 Phytohormones and metabolites – Method development for LCMS-MS quantification

The method for the extraction and quantification of phytohormones and their metabolites was adapted from that of Chiwocha *et al*., (2003), using a HPLC system consisting of an Alliance 2695 separation module(Waters) equipped with a 100 mm x 2.1 mm, 3.5 µm Eclipse XDB C18 column (Agilent), with a 12.5 mm x 2.1 mm, 5 µm Zorbax XDB-C8 guard column (Agilent). The mobile phase was a ternary system comprising acetonitrile (A), de-ionised water (B), and 5% glacial acetic acid in water (C). The gradient was of increasing acetonitrile content, constant glacial acetic acid

concentration of 7 mM (pH 3.4), and an initial flow rate of 0.200 ml min^{-1}. The MS was a quadrupole tandem mass spectrometer (Micromass) outfitted with an electrospray ion source.

Statistical analysis

All statistical analyses were carried out using Genstat for Windows 9th Edition, Version 9.1.0.147 (VSN International Ltd., Herts., UK), and checked by Charles Marshall, Senior Biometrician, Cranfield University. Analysis of variance (ANOVA) was performed on the data specifying a nested treatment structure of a common baseline (observation before curing treatments was the starting point for all three temperature treatments). Least significant difference values (LSD; P=0.05) were calculated from each analysis, for comparison of appropriate treatment means, using critical values of *t* for two-tailed tests.

Part 2: Gemma Chope – CU, Andrew Thompson, John Hammond – WHRI (Objective 2)

Extraction of RNA

Various methods of extracting RNA from onion bulb tissue were tested, however, mostbut were unsuccessful due to the high concentration of carbohydrates in the tissue. The method described below yielded RNA of sufficient quality and quantity.
An initial extraction buffer consisting of TNS (Triisopropylnapthalene-sulfonic acid, sodium salt) and phenol/chloroform was used, followed by a CTAB (Hexadecyltrimethylammonium bromide) extraction to remove carbohydrate contamination. The RNA was then precipitated in NaCl and finally in LiCl, and dissolved in DEPC-treated water.
An extraction buffer consisting of 4% PAS (p-aminosalicylic acid), 1% TNS (Triisopropylnapthalene-sulfonic acid, sodium salt) and 10 mM Tris-HCl at pH 8 was prepared. The required number of DEPC-treated polyallomer tubes (30 ml capacity) were set up in the fume hood, each containing 8 ml extraction buffer, 8 ml phenol/chloroform (50% equilibrated phenol (including 0.1% 8-hydroxyquinolene), 50% chloroform (including 4% iso-amyl alcohol as an anti foaming agent)), and 160 µl β-mercaptoethanol.
Frozen bulb tissue (2 g) was ground in a coffee grinder without allowing the tissue to thaw out. A pre-chilled spatula was used to transfer the tissue to one of the

polyallomer tubes. The tubes were then capped, shaken and stored on ice prior to vortexing for 1 min to mix. The tubes were then centrifuged for 20 min at 15, 000 rpm at 4 °C in a SS34 rotor in a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Du Pont Instruments). Then, 900 µl 3M sodium acetate pH5.4 was added to new 30ml polyallomer tubes. The upper phase was removed and placed into the new tubes, and 0.8 volumes (9.6 ml) of isopropanol was added to each. The tubes were mixed by inverting them and then incubated at -20 °C for at least 1 hr. Following the incubation period, the tubes were centrifuged at 10 10,000 rpm for 15 min at 4 °C. The supernatant was poured off. The tubes were briefly centrifuged, and any remaining supernatant was removed by pipetting. The pellet was dissolved in 400 µl of DEPC-treated water.

Two CTAB (Hexadecyltrimethylammonium bromide) extraction buffers were prepared (2 X CTAB extraction buffer: 2% (w/v) CTAB, 100 mM Tris-HCl pH8, 20mM EDTA and 1.4M NaCl; 1 x CTAB extraction buffer: 1% (w/v) CTAB, 50 mM Tris-HCl pH8, 10mM EDTA). One volume (400 µl) of 2x CTAB extraction buffer was added to the sample, followed by 2 volumes of 1x CTAB precipitation buffer (1600 µl), forming a cloudy solution, which was centrifuged for 20 min at 20 20,000 rpm in a SS34 rotor at 4 °C. The pellet was dissolved in 400 µl DEPC-treated water. This step was repeated, but samples were centrifuged for 10 min at 10,000 rpm. The supernatant was removed, and the pellet was resuspended in 0.5 ml 1.4 M NaCl and transferred to a 2ml Eppendorf tube. Then 2.5 volumes of ethanol (1.25 ml) was added, and the mixture incubated overnight at -20°C.

The samples were centrifuged at 13 13,000 rpm for 30 min at 4 °C. The pellet was resuspended in 1 ml ice-cold 70% (v/v) ethanol, and centrifuged at 13 13,000 rpm for 10 min. The supernatant was removed completely, and the pellet dissolved in 400 µl of DEPC-treated water. The sample was transferred to a new 2 ml tube containing 96 µl of 2M LiCl, and incubated on ice at 4 °C overnight.

The sample was centrifuged at 13 13,000 rpm for 10 min at 4 °C in a microcentrifuge. The precipitate was washed with 3M sodium acetate pH 5.4, and then with 70% (v/v) ethanol. The pellet was dried by leaving open to the air at room temperature. Finally, the pellet was dissolved in an appropriate volume of DEPC-treated water.

Part 3: Kate Downes, Leon Terry – CU (Objective 3)

Plant Material

Onion cvs. Wellington (long-storability) and Sherpa (average storability) were grown at Findlay's and cured at 28°C as described in sections 2.1.1 and 2.1.2.

Experimental design

The experiment was a completely randomised design with three replicates taken from the top, middle and bottom of the field. Each replicate batch was split into five bags per cultivar to be divided between treatments. All bagged onions were buried amongst bulk crates of onions to simulate commercial curing conditions and air flow. There were five treatments for each replicate and cultivar; 1 µl l^{-1} 1-MCP prior to curing, 10 µl l^{-1} ethylene prior to curing, 1 µl l^{-1} 1-MCP post-curing, 10 µl l^{-1} ethylene post-curing and control (no treatment). Gas treatments were conducted in air tight polypropylene chambers (88 cm x 59 cm x 59 cm) with tapped tubes for gas injection by syringe. All gas treatments were administered for 24 hrs at 20°C and untreated control bulbs held at 20°C. After curing all onions were transported to Cranfield University, Silsoe (Beds, UK) for cold storage at 1°C. Diseased or damaged bulbs were removed and the remaining bulbs placed in individual plastic stackable crates and randomly distributed throughout the cold room. Outturns of four bulbs per cultivar, replicate and treatment totalling 120 bulbs per outturn were taken before curing at day 0, after curing at day 42, then at eight week intervals once transferred to cold storage at day 89 and day 147. Two further outturns will be taken on day 203 and 259. Once completed, onion analysis will total 720 bulbs.

Sample Preparation

Sample preparation was as described in section 2.1.3.

Gas analysis

Ethylene and 1-MCP

Following treatment pre- and post-curing, the four onion bulbs were placed in 3 L jars with air tight lids and septum. The jars were sealed for *ca.* 4 hours and gas samples removed with repeated full withdrawal-injection displacements using a 30 ml plastic syringe. The gas sample was analysed for 1-MCP and ethylene using gas chromatography (GC model 8340, DP800 integrator, Carlos Erba Instruments,

Herts., UK) with flame ionisation detector. The four onions were weighed and the concentration of ethylene and 1-MCP calculated as nmoles kg^{-1} h^{-1}.

Respiration rate

Respiration rate analysed according to Chope *et al*., (2007)

Biochemical Analysis

Total flavonoids, total phenolics, total antioxidant activity and pyruvate concentration were measured by absorbance assays. The concentration of non-structural carbohydrates (NSC); glucose, fructose and sucrose, were quantified using HPLC.

Quantification of antioxidants

Antioxidant extraction

Freeze-dried onion powder (150 mg) was mixed with 3 ml of 70:29.5:0.5 methanol : water : hydrochloric acid and vortexed to mix thoroughly. The vials were incubated at 37°C for 1 ½ hours in a shaking water bath vortexing every 15 min. Cooled samples were filtered through 0.2 µm Millex-GV syringe driven filter unit (Millipore Corporation, MA, USA) and stored at -20°C until required.

Total Phenolics, Total Flavonoids and Total Antioxidant Absorbance Assay

Total phenolics were quantified in onion bulb tissue using the Folin-Ciocalteu method (Singleton and Rossi, 1965).

Total flavonoids were quantified by adding 3 ml of sodium hydroxide solution (0.1 M NaOH in 1:1 (v/v) ethanol:water) to 100 µl of onion extract and incubating at room temperature for 10 min. UV absorbance was measured at 420 nm and calibrated against quercetin standards.

For total antioxidants, 50 µl of onion extract was added to 3.6 ml of fresh FRAP (Ferrous Reducing Antioxidant Power) solution (5 ml of 10 mM TPTZ, 5 ml of 20 mM $FeCl_3$ and 50 ml of 300 mM acetate buffer pH 3.6) and incubated at 37°C for 10 min. UV absorbance was measured at 593 nm and calibrated against iron (II) sulphate standards.

Pyruvate analysis

Pyruvate concentration was measured as per final report for HL0164

Quantification of NSC

Onion bulbs were extracted and quantified according to Davis *et al.* (2007) and that detailed in final report HL0164.

Statistical analysis

Statistical analysis was performed as described in section 2.1.6, with modifications. Analysis of variance (ANOVA) was analysed as a nested treatment structure with a common baseline which included the three pre-curing treatments.

Results

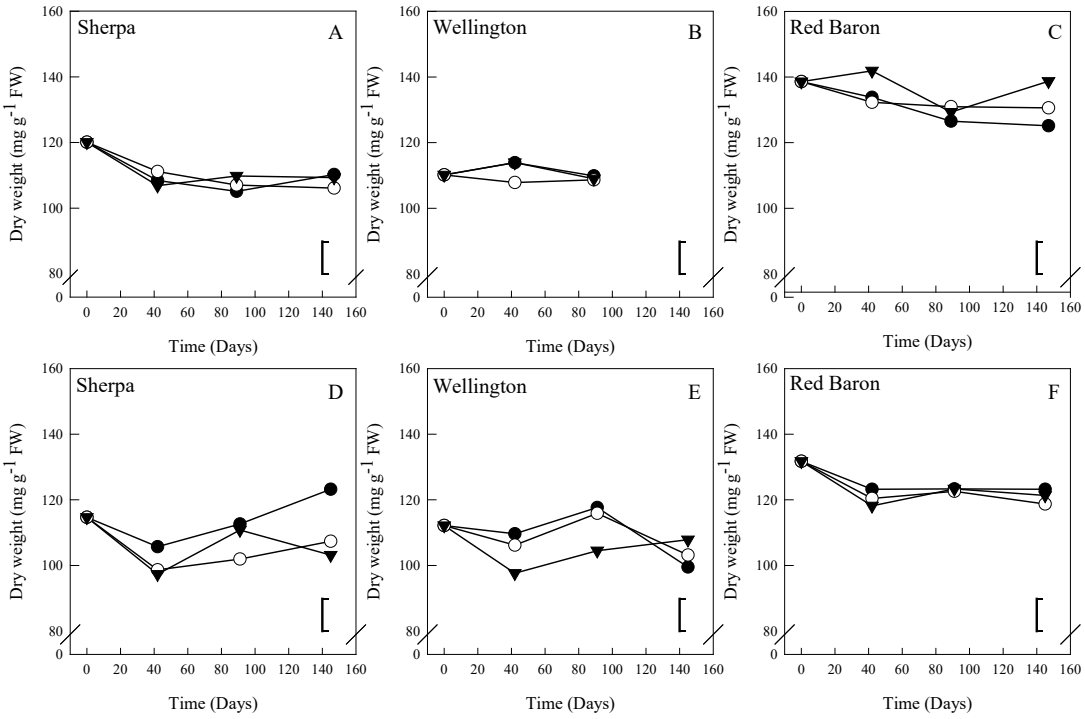
The results presented in this annual report represent data collected up to the current time. The storage season began in September, and the experiment is due to run until May / June, therefore the complete data set in unavailable.

Part 1: Gemma Chope, Leon Terry, CU. (Objectives 1 and 4)

Physical measurements

Dry weight

Onion bulbs cv. Red Baron had a significantly (P>0.001) higher mean dry weight than cvs. Wellington and Sherpa. In addition, the dry weight of bulbs grown at Findlay's was higher (P>0.001) than the dry weight of those grown at Elveden. Overall there was a trend for a decrease in proportion dry weight to occur between the beginning and end of curing (Figure 3.1.1). There was no overall effect of treatment, and generally little change in dry weight after curing.



Proportion dry weight of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's (A, B, C) or Elveden (D, E, F) cured at 20ºC (closed triangles), 24ºC (open

circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

Sprout length

Samples have currently been taken up to 15 weeks cold storage, and no sprout growth has been recorded.

Disease incidence

No incidence of neck rot has been recorded.

Colour

Colour was recorded after curing. There was a main effect of cultivar, site and treatment (P<0.001) on lightness of onions, where the brown cultivars Sherpa and Wellington were lighter in colour (i.e. had a lightness value closer to 100) then the red cultivar, Red Baron. Onions grown at the Findlay's site were lighter than those grown at Elveden, and onions cured at 20ºC were lighter than those cured at 24 and 28ºC, however there was a significant interaction between treatment and cultivar (P<0.001), where lightness of onions cv. Red Baron generally increased with increasing curing temperature, and that of cvs. Sherpa and Wellington decreased (Figure 3.1.2).

Photos below are of samples taken from ABC bulk storage

20°C

24°C

28°C

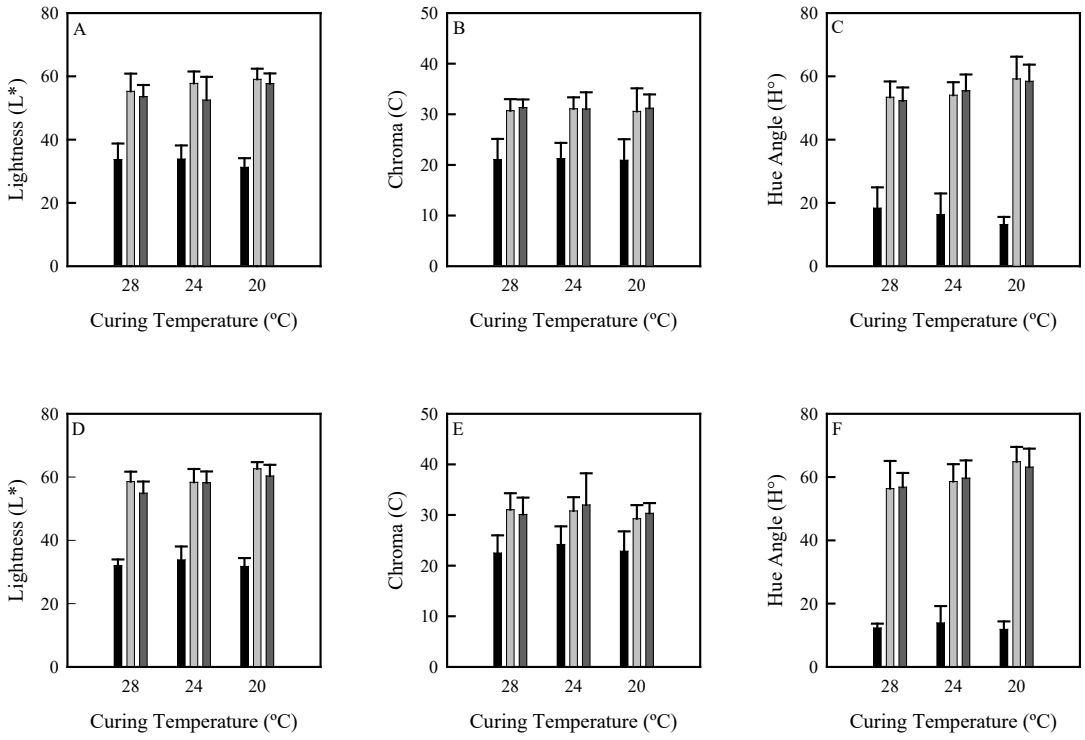


Figure 3.1.2. The lightness, chroma and hue angle of onion cvs. Red Baron (black), Sherpa (light grey) and Wellington (dark grey) cured at 28, 24 or 20ºC for six weeks.

The chroma value recorded was only significantly (P<0.001) affected by cultivar, with the red cultivar having a lower chroma value (more dull colour) than the brown cultivars. The hue angle describes the colour of the skin, and is expressed in degrees where 0º = red, 90º = yellow, 180º = green and 270º = blue. There was a main effect of cultivar (P<0.001), site (P=0.004) and treatment (P<0.001) on hue angle of onions, where the brown cultivars Sherpa and Wellington had a higher hue angle (57.73 and 57.63 respectively) than the red cultivar, Red Baron (14.32). Onions grown at the Findlay's site had a higher mean hue angle than those grown at Elveden, and onions cured at 20ºC were had a higher hue angle than those cured at 24 and 28ºC, however there was a significant interaction between treatment and cultivar (P<0.001), where hue angle of onions cv. Red Baron generally increased with increasing curing temperature, while that of cvs. Sherpa and Wellington decreased.

Biochemical measurements

Pungency

For pyruvate concentration, there were significant main effects of cultivar and site (P<0.001), whereby onions cv. Red Baron were more pungent than onions cv. Sherpa and Wellington and onions grown at the Findlay's site contained more pyruvate than those grown at Elveden (Figure 3.1.3). There was no significant difference in the pyruvate concentration of onions cured at different temperatures; however, in general, there was an increase in pyruvate concentration over time. This effect was more apparent in onions grown at the Findlay's site than at Elveden.

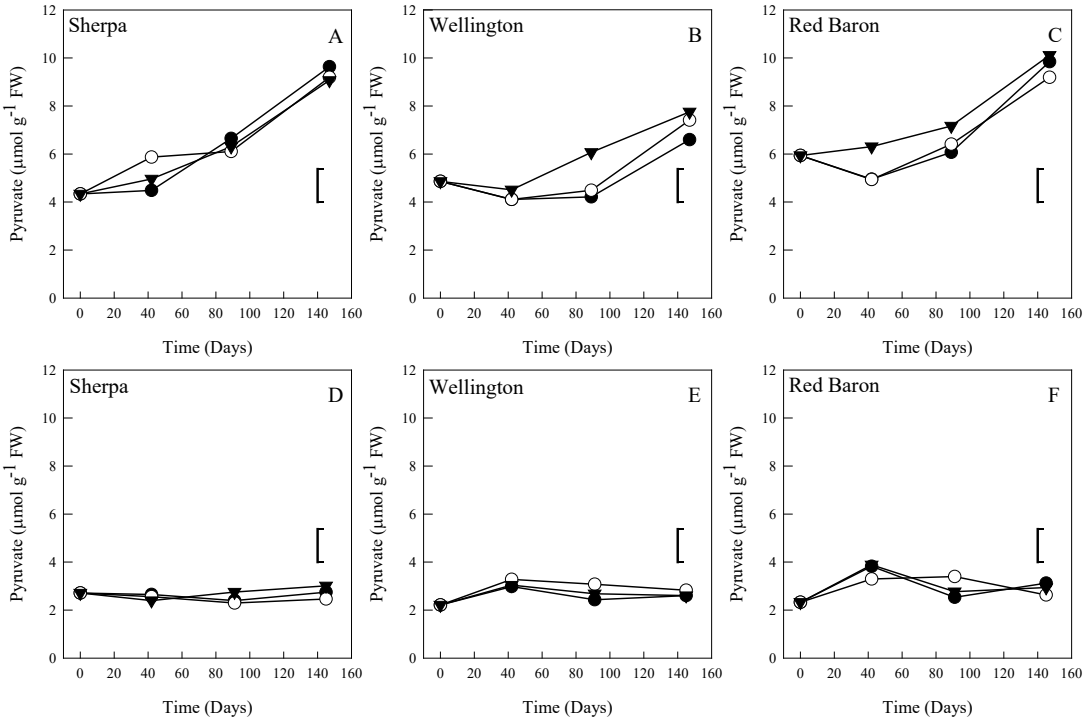


Figure 3.1.3. Pyruvate concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's (A, B, C) or Elveden (D, E, F) cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

Non-structural carbohydrates

When non-structural carbohydrates were measured on freshly harvested onion bulbs there was a significant effect of cultivar (P<0.001) and site (P=0.022) on fructose concentration where the cultivars could be ranked in order of decreasing fructose concentration as follows Sherpa > Wellington> Red Baron, and onions grown at Elveden had a higher fructose concentration than those grown at Findlay's (Table 3.1.1). Similarly there was a significant effect of cultivar (P<0.001) and site

(P=0.001) on glucose concentration, that followed the same pattern as for fructose. Sucrose concentration was not significantly different between cultivars, but there was a main effect of site, where again the sucrose concentration was greater in onions grown at Elveden.

Table 3.1.1. Fructose, sucrose and glucose concentration (mg g^{-1} DW) of freshly harvested onion bulbs cv. Red Baron, Sherpa and Wellington, grown at two different sites, Elveden and Findlay's.

	Elveden			Findlays		
	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
Fructose[A] (mg g^{-1} DW)	35.8	68.7	63.1	30.1	63.5	54.6
Glucose[B] (mg g^{-1} DW)	155.2	203.6	180.5	136.5	188.6	166.2
Sucrose[C] (mg g^{-1} DW)	135.0	115.1	133.1	99.0	113.5	112.3

[A] $LSD_{(0.05)}$ = 9.55; [B] $LSD_{(0.05)}$ = 16.16; [C] $LSD_{(0.05)}$ = 19.03.

Onions grown at Elveden, and that were cured at 20ºC had greater glucose and fructose concentrations than those cured at 24 and 28ºC, but sucrose concentration was not affected by curing temperature (Figure 3.1.4).

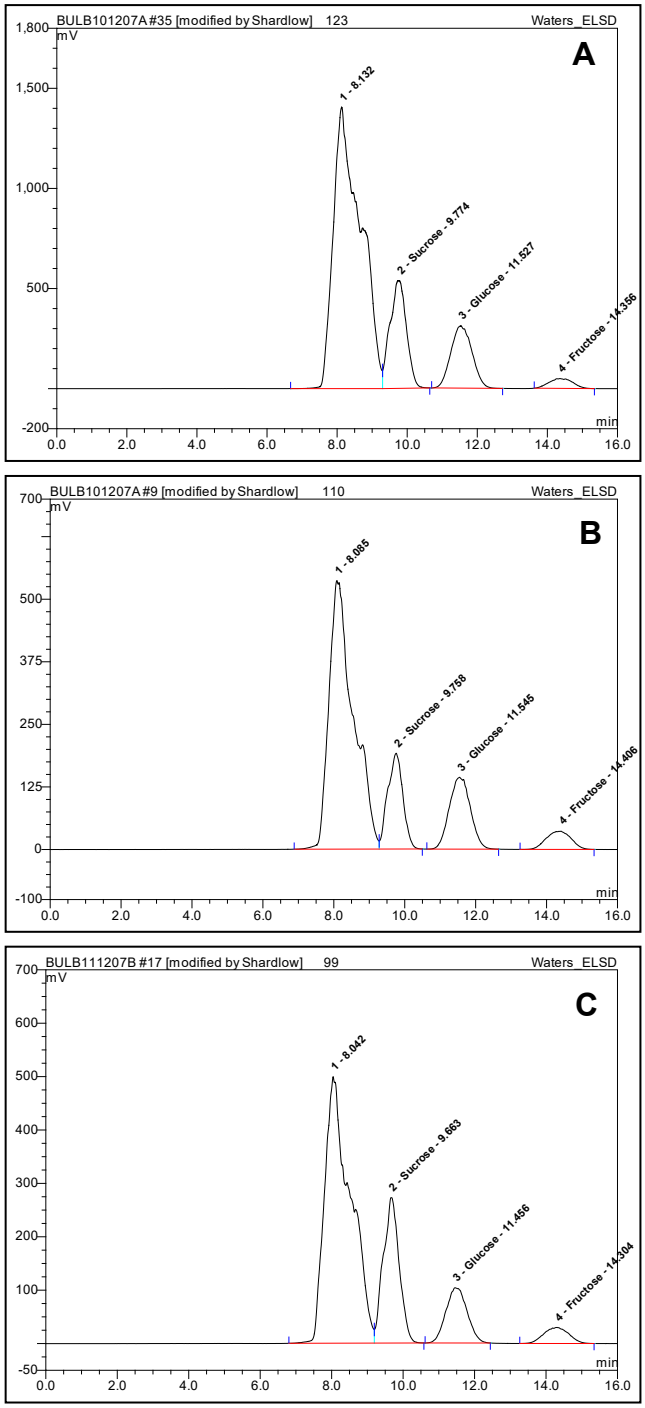


Figure 3.1.4. A typical chromatogram of onion bulb sugars, showing sucrose, glucose and fructose, from onion cv. Red Baron, grown at Elveden, after curing at 20ºC (A), 24ºC (B) or 28ºC (C) for six weeks. Calculated concentrations were; A – 20ºC; fructose 85.87 mg g^{-1} DW, glucose 273.87 mg g^{-1} DW, sucrose 315.56 mg g^{-1} DW, B – 24ºC; fructose 69.39 mg g^{-1} DW, glucose 163.82 mg g^{-1} DW, sucrose 157.17 mg g^{-1} DW, C – 28ºC; fructose 42.27 mg g^{-1} DW, glucose 115.85 mg g^{-1} DW, sucrose 194.50 mg g^{-1} DW.

Onion skin samples from one site – Elveden - were analysed for non-structural carbohydrates, and were found to contain glucose, with smaller concentrations of fructose, and only trace amounts of sucrose (Figure 3.1.5). There was a significant difference between cultivars with Red Baron and Sherpa containing more fructose and glucose than Wellington (Table 3.1.2).

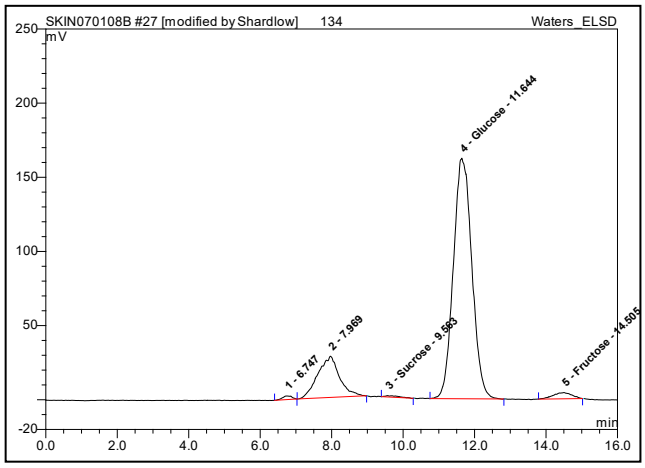


Figure 3.1.5. A typical chromatogram of onion skin sugars, showing sucrose, glucose and fructose, from onion cv. Sherpa, grown at Elveden and cured at 24ºC for six weeks. Calculated concentrations were; fructose - 0.108 mg g^{-1} FW, glucose – 31.083 mg g^{-1} FW, sucrose – trace.

Table 3.1.2. Fructose, sucrose and glucose concentration (mg g^{-1} FW) of skin from onion cvs. Red Baron, Sherpa and Wellington grown at Elveden.

Carbohydrate	Cultivar		
(mg g^{-1} FW)	Red Baron	Sherpa	Wellington
Fructose[A]	2.3	1.3	0.6
Glucose[B]	17.8	26.4	13.1
Sucrose	Trace	Trace	Trace

[A] $LSD_{(0.05)}$ = 1.12; [B] $LSD_{(0.05)}$ = 5.95.

There were no main effects of treatment, but there was an interaction between treatment and cultivar (fructose P=0.07; glucose P=0.012). In general, for cvs. Sherpa and Red Baron, glucose and sucrose concentrations were greater when the

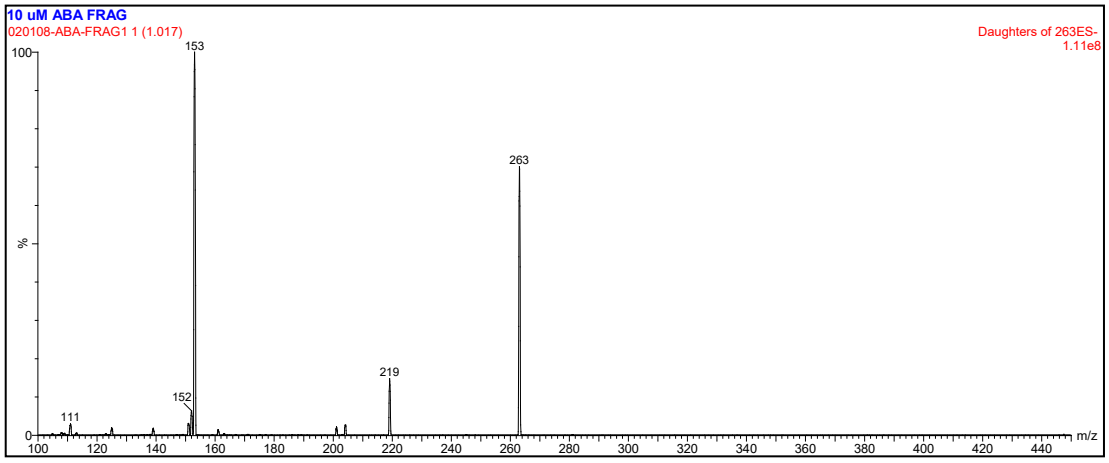
bulbs were cured at 28 ºC than at 24 or 20 ºC, but the opposite was true for cv. Wellington. For cvs. Red Baron and Wellington there was a significant positive correlation between fructose concentration and the hue angle (0.361, P=0.015; 0.328, P=0.028 respectively).

Phytohormones and metabolites – Method development for LCMS-MS quantification

The precursor to product ion transitions for the compounds to be analysed and their internal standards were confirmed (data not shown) from those previously published using pure standard compounds. Examples are shown in Figure 3.1.6. The retention time for each compound was determined (Table 3.1.3).

Figure 3.1.6. The fragmentation pattern for labelled and unlabelled ABA standards. A - Precursor (*m/z* 263.082) and product (*m/z* 152.989) ions of ABA analysed in negative ion mode. B - Precursor (*m/z* 267.177) and product (*m/z* 156.011) ions of ABA analysed in negative ion mode.

A –Abscisic acid (ABA)



B – d_4-Abscisic acid (d_4-ABA)

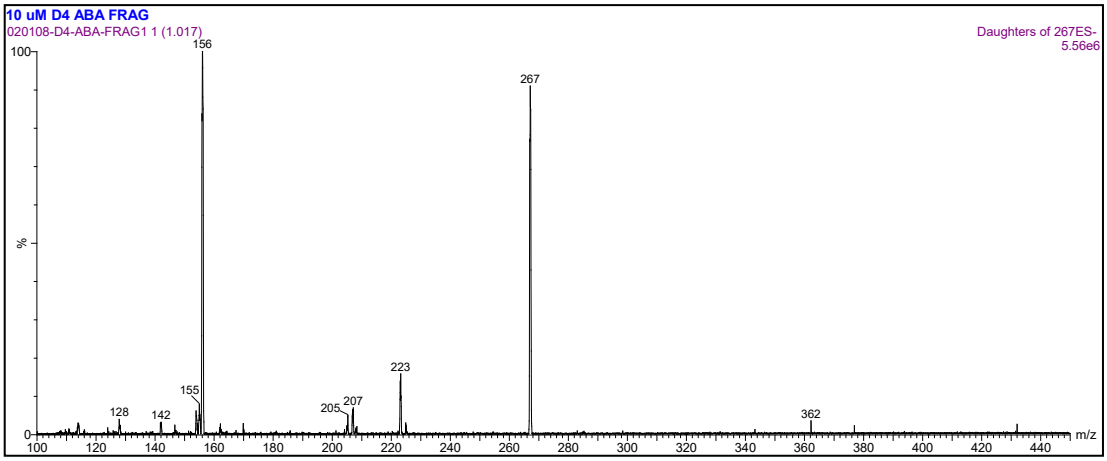


Table 3.1.3. The ionisation mode, characteristic product to precursor ion transition, and retention time for phytohormones and metabolites, along with their respective deuterated internal standards.

Ionisation mode	Compound	Transition	RT (min)	Internal standard	Transition	RT (min)
Positive	Z	220 > 136	9.52	d_3-DHZ	225 > 136	9.17
Positive	ZR	352 > 220	11.30	d_3-DHZR	357 > 225	10.93
Negative	DPA	281 >171	11.89	d_3-DPA	284 > 174	11.84
Negative	IAAsp	289 >132	13.08	d_5-IAA	179 > 135	16.77
Negative	GA_3	345 > 221	13.78	d_2-GA_1	349 > 275	13.86
Negative	GA_1	347 > 273	14.05	d_2-GA_1	349 > 275	13.86
Positive	2iP	204 >136	13.57	d_6-2iP	210 > 137	13.46
Negative	ABA-GE	425 >263	14.29	d_5-ABA-GE	430 > 268	14.13
Negative	PA	279 >139	15.11	d_3-PA	282 > 142	14.96
Positive	IPA	336 >204	15.36	d_6-IPA	342 > 210	15.22
Negative	7`-OH-ABA	279 > 151	15.96	d_4-7`-OH-ABA	283 > 154	-
Negative	IAA	174 > 130	17.03	d_5-IAA	179 > 135	16.77
Negative	ABA	263 >153	18.13	d_4-ABA	267 > 156	17.98
Negative	GA7	329 >223	22.53	d_2-GA_4	333 > 215	22.73
Negative	GA4	331 >213	22.79	d_2-GA_4	333 > 215	22.73

Part 2: Gemma Chope – CU, Andrew Thompson, John Hammond – WHRI (Objective 2)

RNA quantity and quality

The purity and quality of the extracted RNA was determined by running 1 µl aliquots of samples on a chip in a 2100 Bioanalyzer machine (Agilent, CA, USA) according to the manufacturer's instructions (Figure 3.2.1). The Bioanalyzer uses a combination of microfluidics, capillary electrophoresis, and fluorescence to evaluate both RNA concentration and integrity. On the electropherogram, a degraded RNA sample shows a large number of small bands, which appear as a grey smear (Figure 3.2.1A), whereas intact RNA shows two distinct bands that represent the 18S and 28S eukaryotic ribosomal RNA genes (Figure 3.2.1B). Only RNA samples that showed distinct 28S and 18S bands where the relative intensity of the 28S band compared to the 18S band was approximately 2:1.

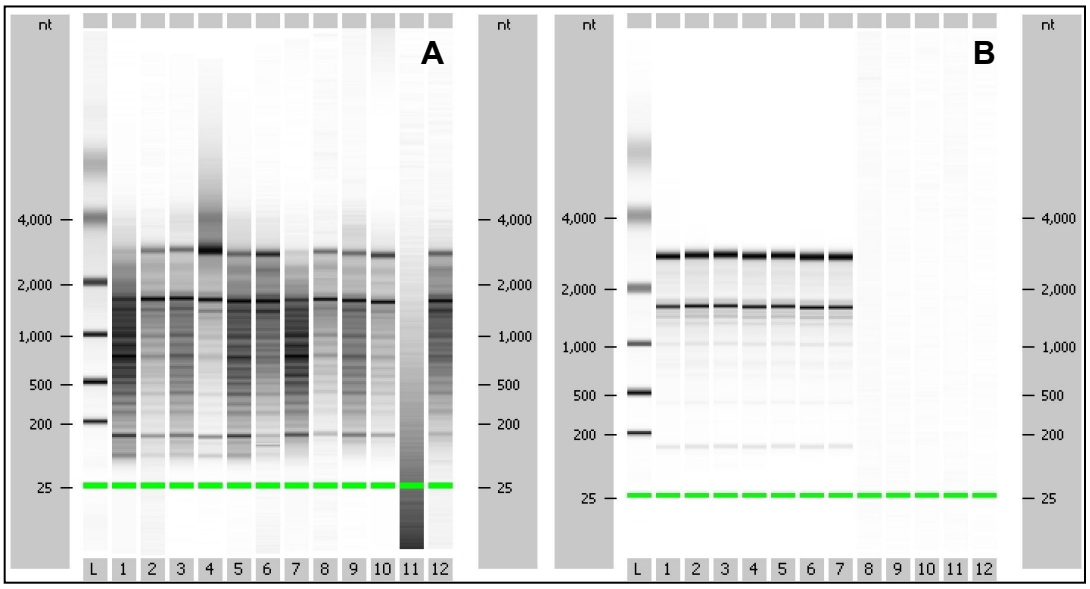


Figure 3.2.1. Typical gel electrophoresis images of sample run on the Bioanalyzer chip showing A – degraded, poor quality RNA, and B – intact, good quality RNA showing the 28S (upper) and 18S (lower) bands.

Selection of genes for inclusion on the microarray

Electronic databases of sequences were mined for information on genes of interest. These genes consisted largely of those from the ABA and ethylene biosynthesis and metabolism pathways (Section 1.1.5). Many of the genes selected for inclusion in the chip are part of multi-gene families, and so first all related protein sequences from *Arabidopsis thaliana* were arranged into a phylogenetic tree, using Lasergene

MegAlign Version 7.1.0 (44) software (DNAStar, Madison, USA) to determine whether genes of a similar function grouped together. The protein sequence of a gene with known function in *Arabidopsis thaliana* was BLAST searched (Basic Local Alignment Tool) using the NCBI (National Center for Biotechnology Information) web site (www.ncbi.nlm.nih.gov). An example is shown in Figure 3.2.2, where the genes with proven NCED function formed one group, while those with other carotenoid cleavage function formed another group. This process was repeated to include sequences from rice (*Oryza sativa*) (Figure 3.2.3) and finally all sequences from monocot species in the database (Figure 3.2.4), these species were rice, crocus, maize (*Zea mays*), barley (*Hordeum vulgare*) and orchid (*Oncidum*).

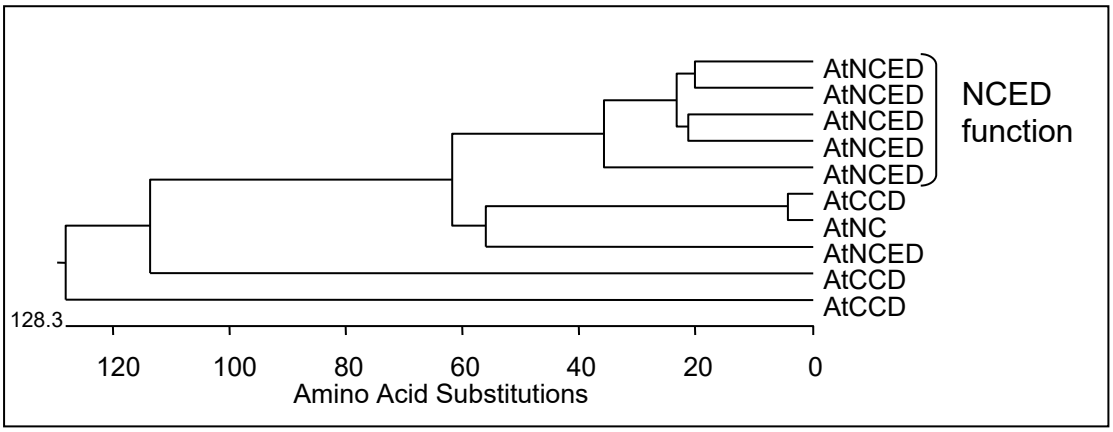


Figure 3.2.2. Phylogenetic tree created using Clustal W alignment.

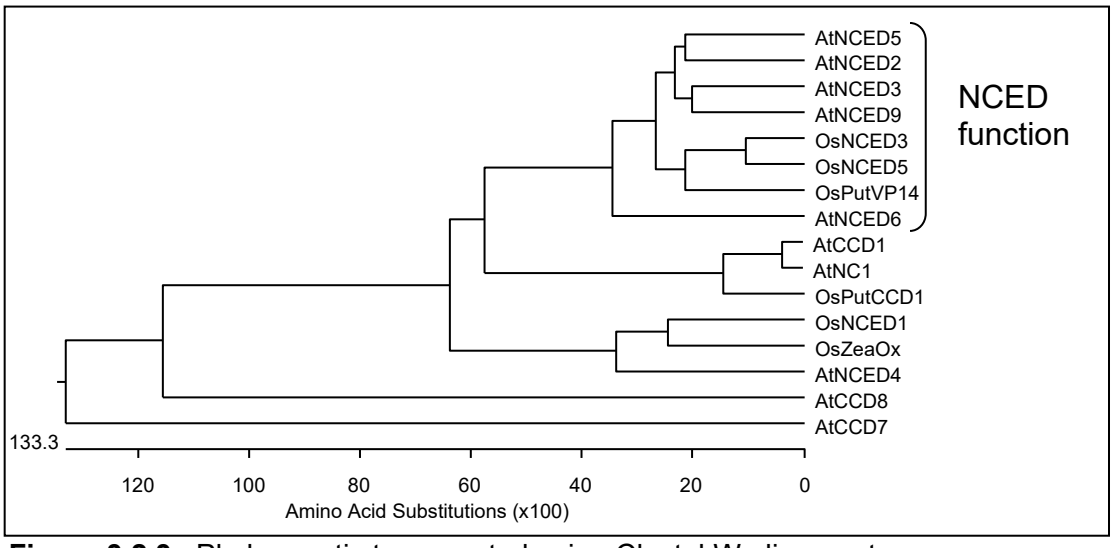


Figure 3.2.3. Phylogenetic tree created using Clustal W alignment.

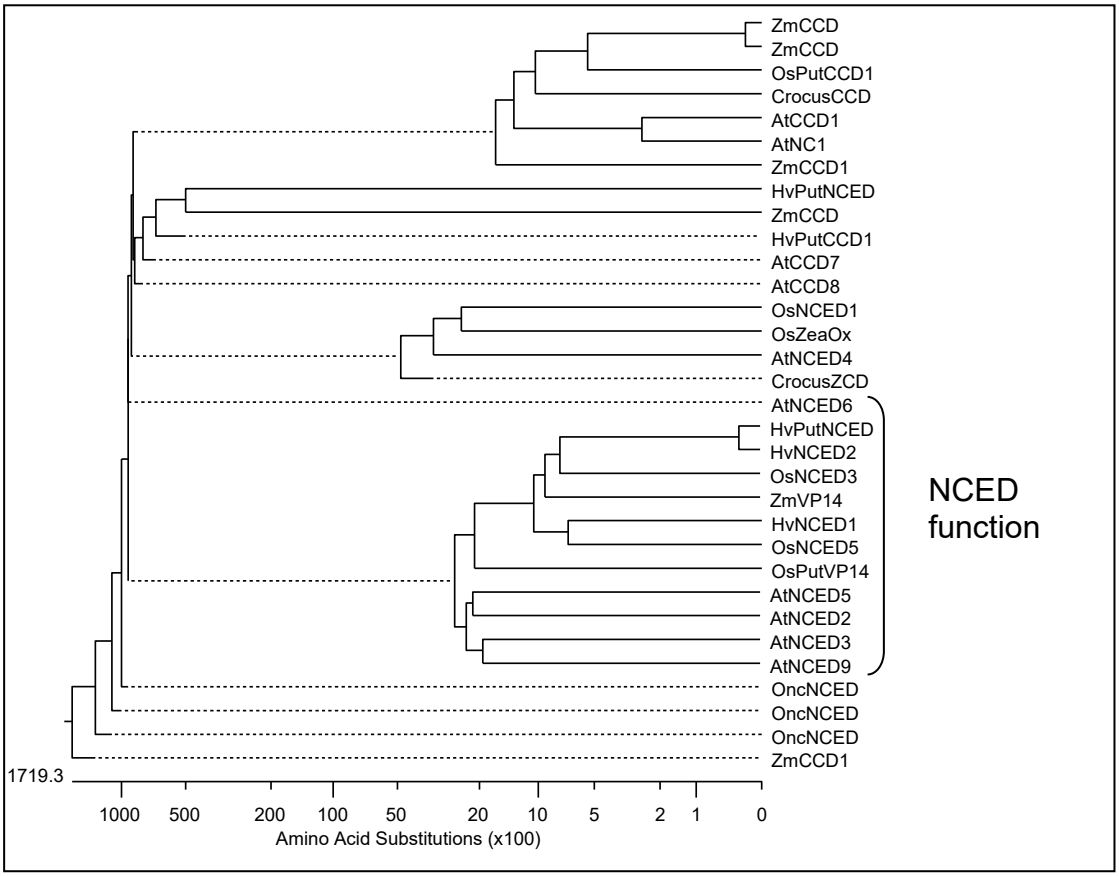


Figure 3.2.4. Phylogenetic tree created using Clustal W alignment.

The protein and mRNA sequences for all the monocot sequences with known NCED function were aligned and a consensus sequence produced. In the case of NCED genes the protein and nucleotide sequences were well conserved. Primers were designed for the conserved regions. Primers used to amplify NCED genes from other species were also used (Table 3.2.1). cDNA was created from the extracted RNA samples using Thermoscript RT-PCR system for first-strand cDNA synthesis (Invitrogen). The primers were then used to amplify the cDNA in a PCR reaction using high fidelity DNA polymerase (Platinum DNA Polymerase, Invitrogen).

Table 3.2.1. Primer sequences to amplify NCED genes from onion cDNA.

Primer Pair Sequences (5' to 3') (F = forward; R = reverse) (S = C/G; Y = C/T; N = A/G/C/T, R = A/G)	**Source**	**Target Species / Gene**
F - GAC GTC ATC AAG AAG CCT TAC C R - ATG TCA TCA AGA AGC CGT ACC TC	Millar *et al*. (2006)	Barley NCED
F - CTC GAA TCG ATC GGA CCA GCT CT R - GTG ATG AGT AAC CGC CGC TAA CTG	Chono *et al*. (2006)	Barley NCED1
F - CTC TCT CGC AAC AAA ACC CAC G R - CTG GCA ACT TCC TCT TTC CAT GTC C	Chono *et al*. (2006)	Barley NCED2
F - AGT TGT TGT GTC ACC CAG TCC AG R - CAC GCA CCG ATA GCC ACA	Voisin *et al*. (2006)	Maize NCED/VP14
F - TTY GAY GGN GAY GGN ATG GT R - ACN SCR AAR TCR TGC ATC AT	Burbridge *et al*. (1999)	Tomato NCED
F - GCS GGS CAC CAC CTT STT CG R - GTC GTG GAT CAT SGT SGG CTG STC	Chope	Various monocot species NCED protein consensus
F - CGT CAT CAA GAA GCC GTA CC R - GTT CCA GAG GTG GAA GCA GA	Chope	Various monocot species NCED mRNA consensus
F - GTT CAA GCT CCA GGA GAT GC R - GCC GTA CTC GAA CTT GGT GA	Chope	Various monocot species NCED mRNA consensus

Part 3: Kate Downes – CU (Objective 3).

Dry weight

No significant differences in dry weight were found between onion bulbs cv. Sherpa and Wellington as both decreased in dry weight during the 47 days in cold storage (Figure 3.3.1). Only cv. Sherpa significantly decreased in dry weight during the 42 days curing ($P< 0.001$). No significant differences between treatments were found at day 0 and 42. At 89 days only the control onion bulbs and the post-curing ethylene treated onions showed significant differences in dry weight for cv. Sherpa ($P< 0.001$). At 89 days there were significant differences between the dry weights of treated onion bulbs cv. Wellington however none of these were significantly different from the control ($P< 0.001$).

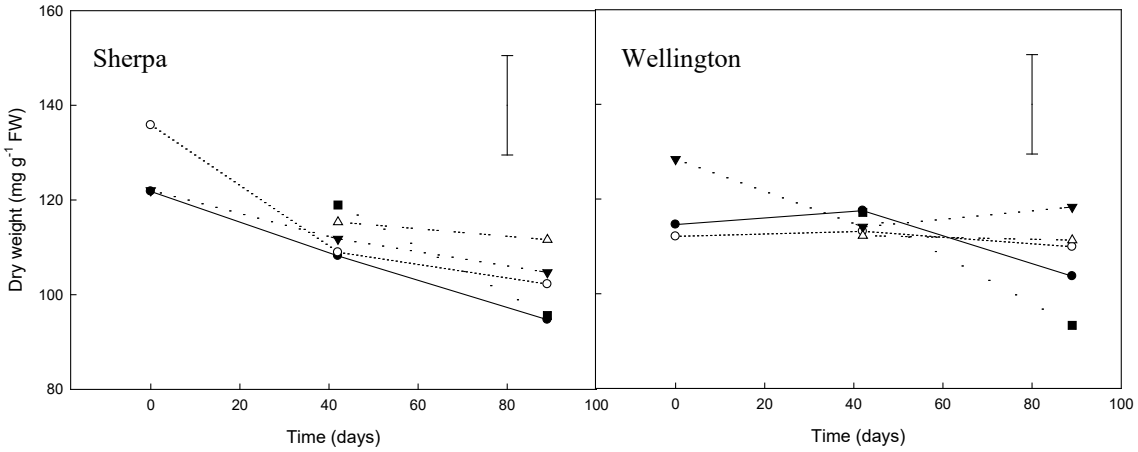


Figure 3.3.1. Changes in dry weight before curing (0 days), after curing (42 days) and during storage at 1°C in untreated onion bulbs (control, ●) and onion bulbs treated before curing with 10 µl l^{-1} ethylene (○) or 1 µl l^{-1} 1-MCP (▼) and after curing with 10 µl l^{-1} ethylene (△) or 1 µl l^{-1} 1-MCP (■) for 24 hrs; n = 12; LSD bars (P= 0.05) are shown.

Ethylene and 1-MCP evolution

Comparisons were made between gas evolution of bulbs treated before curing and those treated after curing to identify links between the gas permeability of the onion skin and the curing process. Onions cv. Sherpa evolved 24% higher concentrations of ethylene gas after the pre-curing treatment (10.13 nmol kg^{-1} hr^{-1}) than after the post-curing treatment, (8.183 nmol kg^{-1} hr^{-1}) (Table 3.3.1). Onions cv. Wellington also yielded 12.5% higher levels of ethylene gas after the pre-curing treatment (9.064 nmol kg^{-1} hr^{-1}) than after the post-curing treatment (8.060 nmol kg^{-1} hr^{-1}). Overall onions treated before curing with ethylene evolved 18% more ethylene than those treated after curing with ethylene. Onions cv. Sherpa evolved higher concentrations of ethylene gas than cv. Wellington for both pre- and post-curing treatments.

Onions cv. Sherpa yielded 1.902 nmol kg^{-1} hr^{-1} of 1-MCP after the pre-curing 1-MCP treatment however no detectable gas was measured after the post-curing treatment. Onions cv. Wellington evolved higher concentrations of 1-MCP gas after the pre-curing treatment (1.630 nmol kg^{-1} hr^{-1}) than after the post-curing treatment (0.947 nmol kg^{-1} hr^{-1}).

None of the control onions produced any ethylene or 1-MCP. Onions treated with ethylene or 1-MCP before curing did not produce any ethylene or 1-MCP gas after

curing (data not shown). Only onion bulbs which were treated immediately before the treatments produced any ethylene or 1-MCP.

Table 3.3.1. Ethylene and 1-MCP gas evolution immediately after pre- and post-curing treatments with 10 µl l^{-1} ethylene and 1 µl l^{-1} 1-MCP.

		Treatment		
Cultivar	Gas evolution (nmol kg^{-1} hr^{-1})	Control	Before curing	After curing
Sherpa	Ethylene	0	10.13	8.183
	1-MCP	0	1.902	0
Wellington	Ethylene	0	9.064	8.060
	1-MCP	0	1.630	0.947

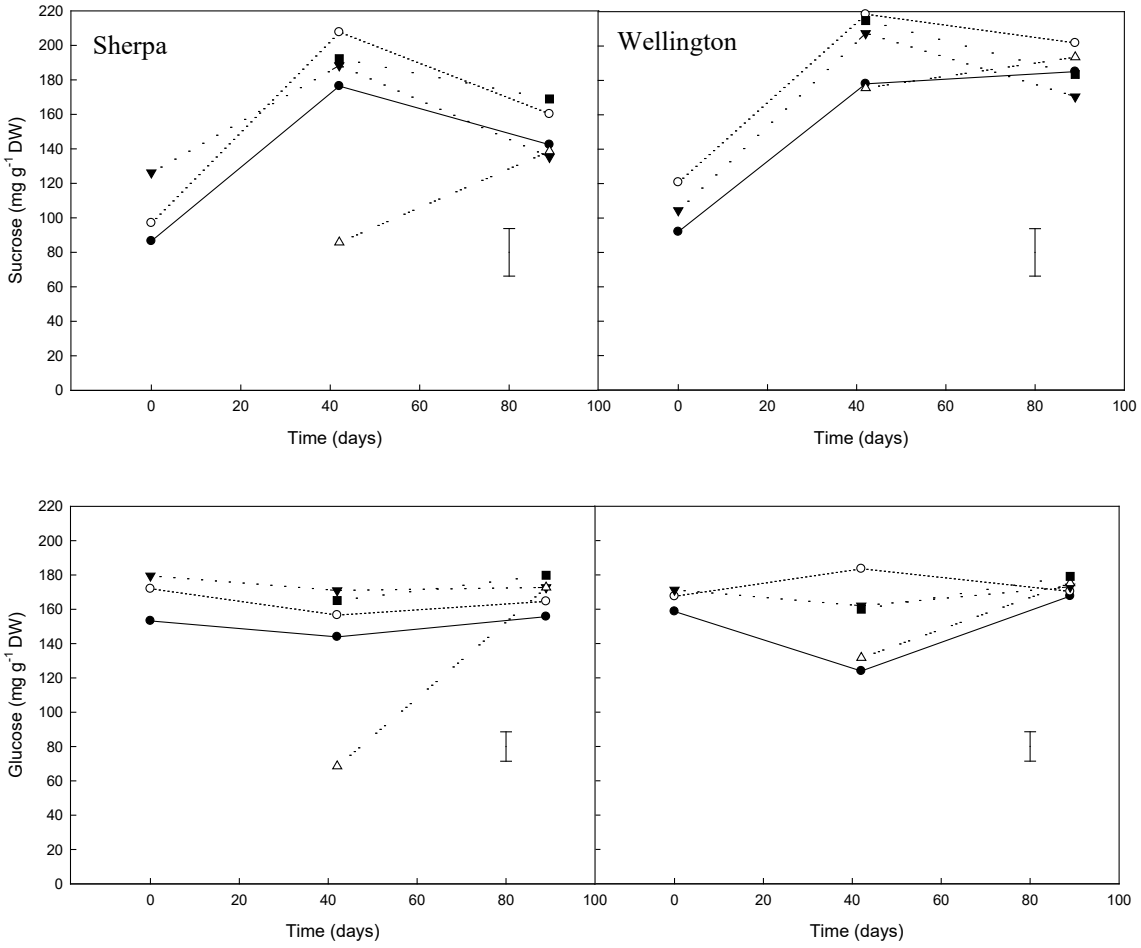
Fructose, glucose and sucrose

To compare the effect of ethylene and 1-MCP on carbohydrate profile, glucose, fructose and sucrose (a disaccharide of glucose and fructose) were measured (Figure 3.3.2). The only significant differences found between cultivars was at 42 days immediately after curing for glucose and sucrose due to the very low concentrations of 68.4 and 85.7 mg g^{-1} DW, respectively found in onions treated with ethylene after curing for cv. Sherpa only. The greatest changes between sugars pre-curing and post-curing were seen in sucrose concentration which doubled in concentration for both cultivars and was significantly different over time ($P<0.001$). When transferred to storage at 1 °C, the sucrose concentration then decreased significantly between 42 and 89 days in onion bulbs cv. Sherpa ($P<0.001$) however this was not significant for cv. Wellington.

Overall for both cultivars, the mean glucose concentration decreased significantly from 167 mg g^{-1} DW down to 146.6 mg g^{-1} DW then increased up to 171.1 mg g^{-1} DW ($P<0.001$) after 89 days. After the pre-curing 1-MCP and ethylene treatments, onions cv. Sherpa had significantly higher concentrations of glucose than the post-curing ethylene and 1-MCP treated onions ($P=0.004$), this was not significant for onions cv. Wellington. Significant differences in glucose concentrations were found between treatments at 42 days with post-curing ethylene treated onions cv. Sherpa showing very low concentrations of glucose at 68.5 mg g^{-1} DW ($P<0.001$). Post-curing ethylene treated onions cv. Wellington also showed lower levels than the other

treatments at 42 days however this was not significantly different from the control. At 89 days no significant differences were found between cultivar or treatment with mean glucose concentrations for onions cv. Sherpa and Wellington at 169.1 and 173.1 mg g^{-1} DW, respectively.

Similarly, a difference in fructose concentration between treatments was observed at 42 days ($P<0.001$) with no significant differences in fructose concentration between treatments at 0 or 89 days after harvest. The only significant differences over time were during cold storage between 42 and 89 days with a mean increase in fructose concentration from 51.8 – 156.8 mg g^{-1} DW ($P<0.001$).



Sherpa Wellington

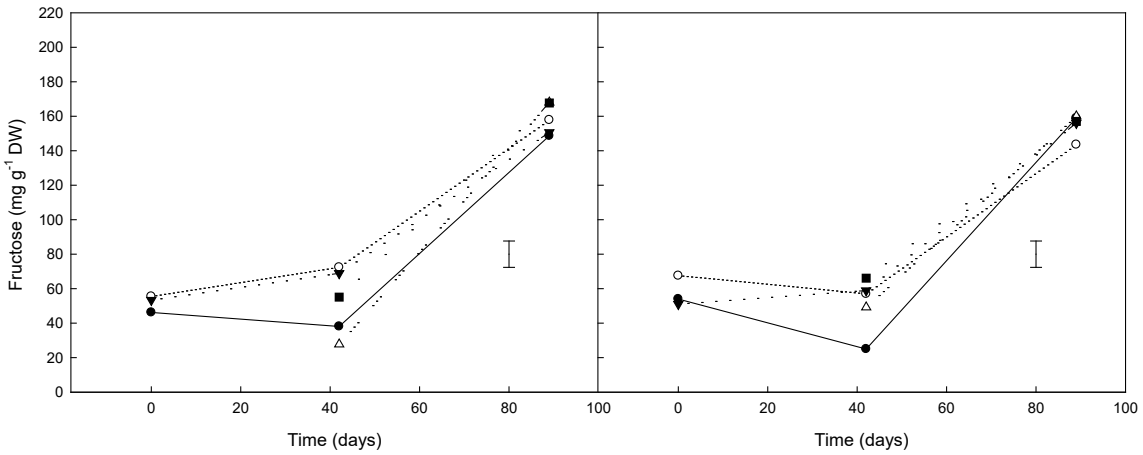


Figure 3.3.2. Changes in sucrose, glucose and fructose concentrations before curing (0 days), after curing (42 days) and during storage at 1°C in untreated onion bulbs (control, ●) and onion bulbs treated before curing with 10 µl l^{-1} ethylene (○) or 1 µl l^{-1} 1-MCP (▼) and after curing with 10 µl l^{-1} ethylene (△) or 1 µl l^{-1} 1-MCP (■) for 24 hrs; n = 12; LSD bars (P=0.05) are shown.

Total phenolics, flavonoids and antioxidant capacity

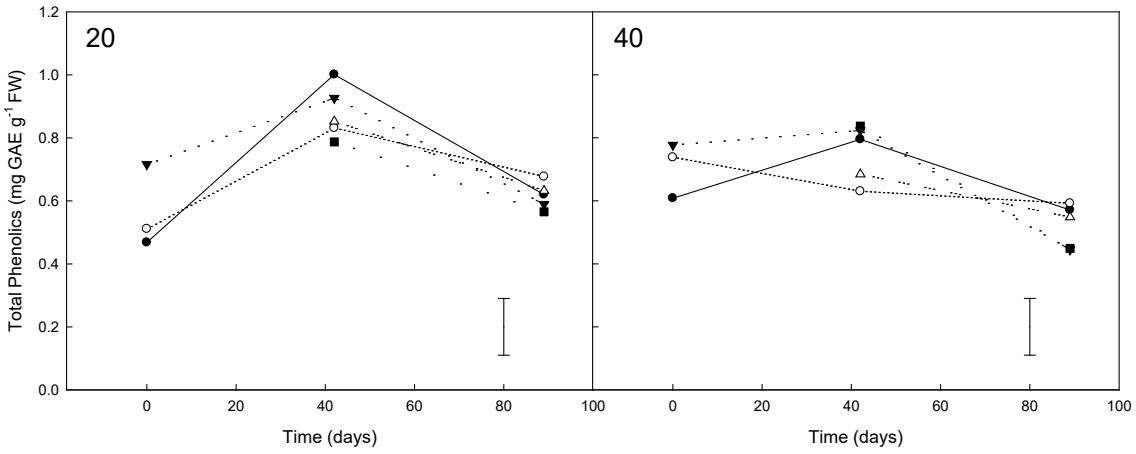
There was a main effect of time whereby the total phenolic concentration of onions increased significantly during curing then decreased significantly once transferred into cold store (P<0.001). Differences between the two cultivars were also found to be significant over time with cv. Wellington showing less dramatic change in the increase during curing and decrease during cold storage (Figure 3.3.3). Significant differences between treatments were only found at day 0 between pre-curing 1-MCP treated onion bulbs, cv. Sherpa and the control bulbs however this was not significant for onions cv. Wellington (P=0.006).

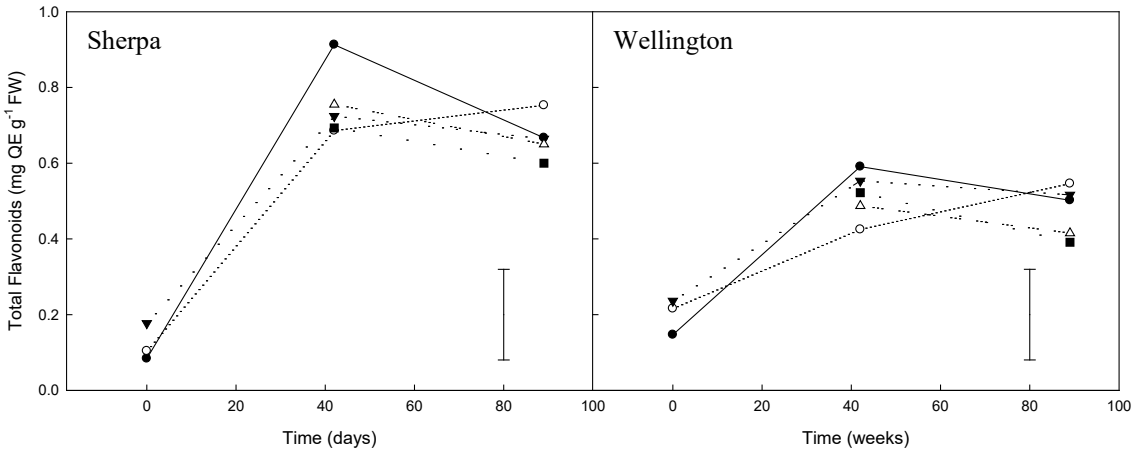
Total flavonoid concentrations in all onions increased significantly during the 0 – 42 days of curing (P<0.001) however the small decrease during cold storage was not significant (Figure 3.3.3). Significant differences were found between the two cultivars with cv. Wellington showing less dramatic changes than cv. Sherpa as seen for total phenolics (P<0.001). Onions cv. Sherpa appear, so far, to have higher levels of total flavonoids than cv. Wellington. No significant differences in total flavonoid levels were found between treated and untreated onions.

There was a main effect of time where the total antioxidant capacity of onions cv. Sherpa increased from 3.19 µM Fe^{2+} g^{-1} FW at day 0 to 4.73 µM Fe^{2+} g^{-1} FW at day 42 (Figure 3.3.3). However onions cv. Wellington decreased from 5.03 µM Fe^{2+} g^{-1}

FW at day 0 to 3.84 µM Fe^{2+} g^{-1} FW at day 42 causing a significant interaction between cultivars and time (0 – 42 days only) (P<0.001). Pre-curing 1-MCP treated onions cv. Sherpa showed significantly higher total antioxidant capacity than the other treatments (P=0.009) however this was not significant at 42 or 89 days.

Overall the total flavonoids, phenolics and antioxidant capacity of the onion bulbs behaved similarly with an increase from day 0 to day 42 then a subsequent decrease during cold storage.





Sherpa Wellington

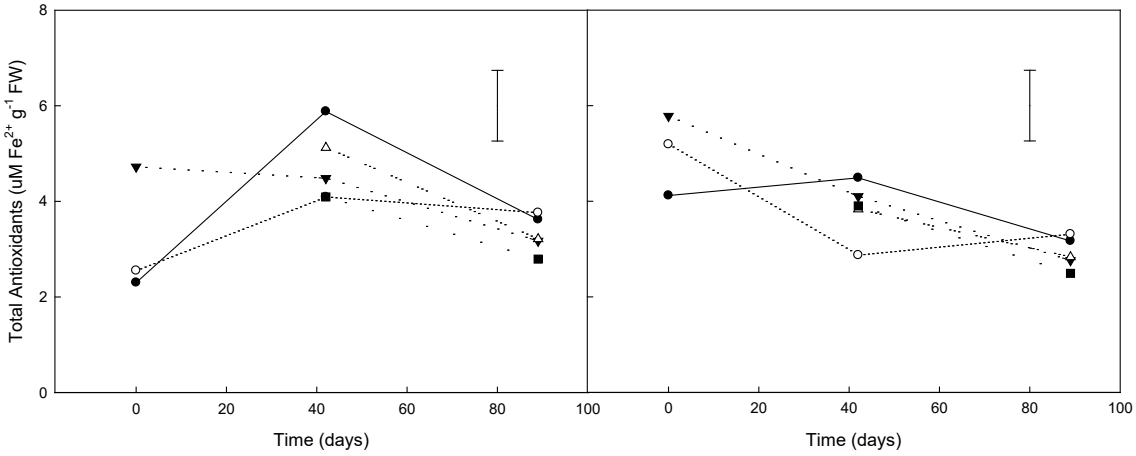


Figure 3.3.3. Changes in total phenolics, total flavonoids and total antioxidant capacity concentrations before curing (0 days), after curing (42 days) and during storage at 1°C in untreated onion bulbs (control, ●) and onion bulbs treated before curing with 10 µl l^{-1} ethylene (○) or 1 µl l^{-1} 1-MCP (▼) and after curing with 10 µl l^{-1} ethylene (△) or 1 µl l^{-1} 1-MCP (■) for 24 hrs; n = 12; LSD bars (*P* = 0.05) are shown.

Respiration rate

The respiration rate of cured onions was significantly lower than that of freshly harvested bulbs (P<0.001) (Figure 3.3.4). There was a main effect of cultivar on the respiration rate of onions where cv. Sherpa had a higher mean rate than cv. Wellington (P<0.001). Pre-curing ethylene treated onions showed a significantly higher respiration rate than the control at time 0 for both cultivars. Additionally at 42 days the post-curing ethylene treated onions showed significantly higher respiration rate than the control for both cultivars (P<0.001). At day 0 1-MCP treated onions cv. Sherpa showed higher respiration rate than untreated onions but significantly lower than the ethylene treated onions (P<0.001). For cv. Wellington the 1-MCP treated onions were not significantly different from the control (P<0.001).

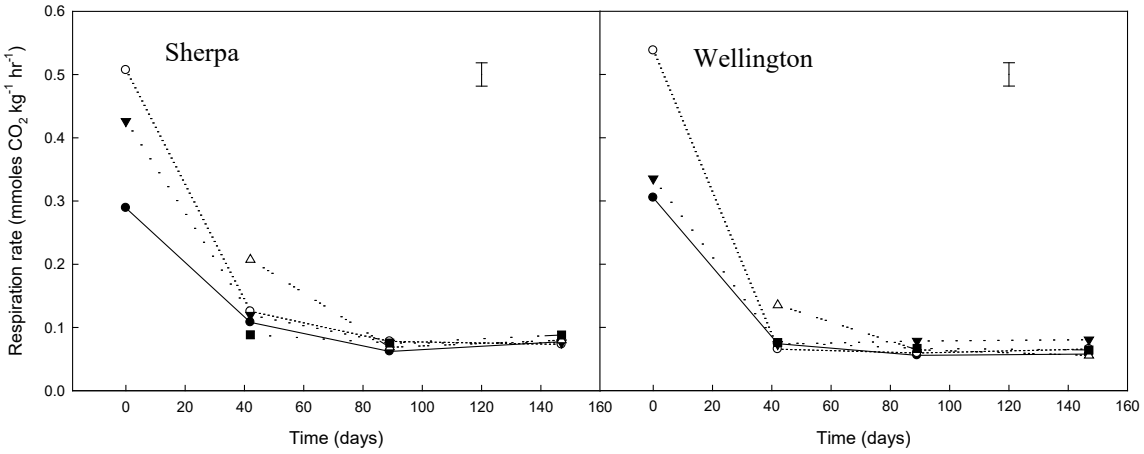


Figure 3.3.4. Changes in respiration rate before curing (0 days), after curing (42 days) and during storage at 1°C in untreated onion bulbs (control, ●) and onion bulbs treated before curing with 10 µl l^{-1} ethylene (○) or 1 µl l^{-1} 1-MCP (▼) and after curing with 10 µl l^{-1} ethylene (△) or 1 µl l^{-1} 1-MCP (■) for 24 hrs; n = 12; LSD bars (P=0.05) are shown.

Pyruvate concentration

Pungency is measured in terms of pyruvate concentration. When onions cells are disrupted pyruvate is produced from the hydrolysis of alk(en)yl cysteine sulphoxides (ACSOs) by the enzyme alliinase (Block, 1992). There was a main effect of cultivar and time on pyruvate concentrations of onions (P<0.001) where cv. Sherpa showed higher mean values at all time points except at 42 days (Figure 3.3.5). At 0, 42 and 147 days significant differences in pyruvate concentration were found between treatments (P<0.001). At 147 days both cultivars showed greater variation between treatments however, although the post-curing 1-MCP treated onions cv. Wellington were significantly different to the other treated bulbs (P=0.002) there was no significant difference from the control.

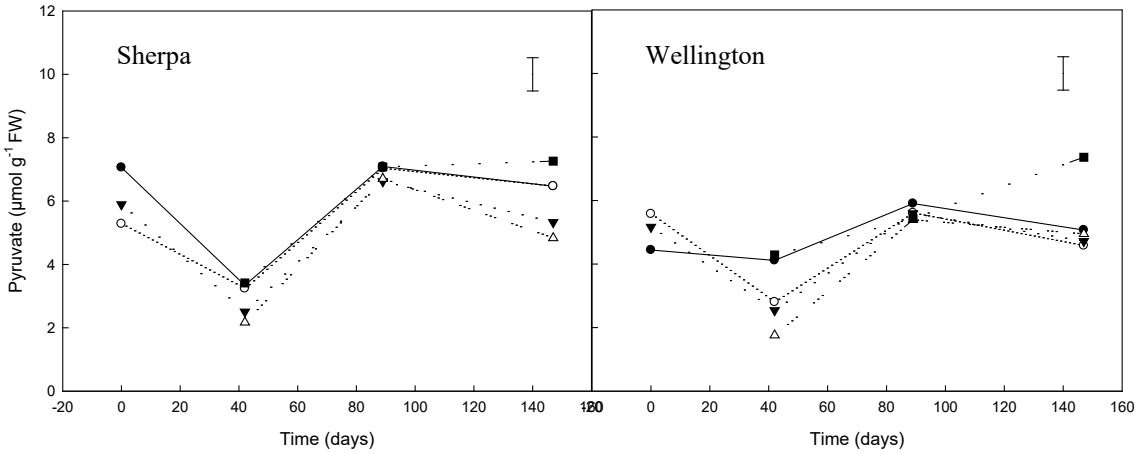


Figure 3.3.5. Changes in pyruvate concentration before curing (0 days), after curing (42 days) and during storage at 1°C in untreated onion bulbs (control, ●) and onion bulbs treated before curing with 10 µl l^{-1} ethylene (○) or 1 µl l^{-1} 1-MCP (▼) and after curing with 10 µl l^{-1} ethylene (△) or 1 µl l^{-1} 1-MCP (■) for 24 hrs; n = 12; LSD bars (P=0.05) are shown.

Discussion

Part 1: Gemma Chope, Leon Terry - CU (Objectives 1 and 4)

The proportion dry weight of freshly harvested onion bulbs was less than that of cured bulbs, this finding is supported by Hansen (1999) on onions cv. Hyduro and Hyton cured at 24 ºC for 5 weeks. This is perhaps counter-intuitive as moisture is lost during the curing process. However, the moisture loss is greater from the thin, outer skins than from the inner scales. Samples of bulb tissue were taken from the bulb with the outer scales removed; therefore the loss of moisture in this part of the bulb would not be reflected in the dry weight measurements taken. The reduction in proportion dry weight may therefore be due to increased respiration rate caused by the high temperatures during curing (Hurst *et al*., 1985). The proportion dry weight was not affected by treatment and so this indicates that curing at a lower temperature does not affect this parameter.

Although statistically significant differences in the chroma and hue angle of onions when measured after curing at different temperatures could be detected, these differences were not likely to be sufficient to be detected in isolation or to have a negative effect on consumer selection. It was expected that a lower curing temperature may have resulted in an unacceptable skin finish. The effect of curing temperature on pungency of onion bulbs was not known, but we have shown that the pyruvate concentration of onions was not affected by curing temperature. Differences between the sites in terms of pungency, dry matter and NSC concentrations are likely to be due to the differences between the growing conditions at the two sites, including factors such as soil type (including sulphur content) and the prevailing weather. For example, more water can be held in sandy clay loam soils (as at Findlay's) than in sandy soil (as at Elveden). In addition, onions were harvested at 80% fall-over from Findlay's and at 100% fall-over from Elveden, and Hansen (1999) found that proportion dry matter was greater (although not significantly) in onions cv. Hyduro and Hyton harvested at 40% and 80% fallen tops, than when harvested at 1% and 100%. Hansen (1999) explains the decrease in dry matter in terms of the higher contribution of outer leaves to the total mass of larger bulbs that result from a longer growing period. The outer leaves have a lower dry matter content than the inner leaves, which means that dry matter content decreases during growth.

Onions with a higher proportion of dry matter also tend to be more pungent, and, by definition, contain higher concentrations of other compounds such as non-structural

carbohydrates (Galmarini *et al*., 2001). However, the concentration of fructose, glucose and sucrose was higher in onions grown at Elveden. This is explained by the fact that onions with a higher dry matter content tend to accumulate carbohydrates in the form of large polysaccharides called fructans. For onions grown at Elveden, fructose and glucose were present in higher concentrations in onions cured at 20 ºC than at 24 or 28 ºC. This may be explained by the fact that a higher respiration rate would be expected at the higher temperatures.

Part 2: Gemma Chope, Andrew Thompson – CU, John Hammond – WHRI (Objective 2)

The next stage in the process will be to run the products of PCR reactions prepared using primers designed to amplify various genes from onion on an electrophoresis gel. The pattern of DNA bands will then be visualised by staining the DNA on the gel using ethidium bromide, and then viewing by UV transillumination. Any bands that correspond with the expected product size from any of the primer pairs will be excised from the gel and sequenced. The sequences obtained will then be compared to the monocot consensus sequence.

Part 3: Kate Downes, Leon Terry – CU (Objective 3)

Pre-cured treated onions produced more ethylene and 1-MCP than the post-cured treated onions possibly due to the curing process reducing the permeability by drying the skin and sealing the bulb. The curing process does appear to reduce ethylene and 1-MCP absorption therefore pre-curing application may improve the efficiency of gaseous treatments. Improving uptake efficiency could help to lower costs by reducing the quantity of ethylene or 1-MCP required to achieve sprout suppression.
A lower concentration of ethylene gas was released from onions cv. Wellington compared to the average-storing onions cv. Sherpa. Wellington is a long storing cultivar with thicker skin which may be less gas permeable compared to average storing or low pungency onions. It may therefore be more important that thick skinned cultivars such as Wellington be treated before curing to aid absorption but less important for the thinner skinned sweet onions such as cv. SS1
Due to this reduction of moisture in the skin it would be expected that dry weight would increase. However, dry weight was found to significantly decrease overall during curing and storage. Our analysis of dry weight does not include the skin which is removed during processing, as all sampling is taken from the edible part of

the bulb. The small decrease in dry weight may also be the result of a decrease in dry mass or an increase in bulb water content due to respiration. Chope *et al.* (2007b) discovered a similar decrease in dry weight in sweet onion cv. SS1 stored at 4 °C however this reduction in dry weight was prevented by 1-MCP.

The overall reduction in dry weight may have been caused by the breakdown and metabolism of fructans and sucrose. The reduction in sucrose content and increase in fructose and glucose content as affected by 47 days in cold store at 1°C agrees with Chope *et al*. (2007c), who reported an increase in monosaccharide to disaccharide ratio throughout storage suggesting that sugars are metabolised for sprout development. The carbohydrate profile of onions cv. Sherpa and Wellington are typical of other average- and long-storing onions. Salama *et al*. (1990) found that after 5 weeks in storage at 0°C, onions cv. Sentinel showed increases in fructose concentrations from 80 – 140 mg g^{-1} DW and glucose from 220 – 250 mg g^{-1} DW and a decrease in sucrose from 160 – 140 mg g^{-1} DW. Following these initial changes, glucose, fructose and sucrose concentrations remained stable for the next 5 weeks of storage.

The greatest differences between treatments for all NSCs were found after curing at 42 days. At 89 days, after 47 in cold storage, no treated onion bulbs differed significantly from the untreated bulbs in terms of sugar concentrations. This is consistent with previous data on onion bulbs cv. SS1 which at 4°C showed no significant change in carbohydrate profile between those treated with 1-MCP for 24 hrs and untreated bulbs.

The increase in sucrose content during curing may be due to the onset of dormancy. Immediately after harvest the enzyme sucrose synthase which converts sucrose to glucose and fructose has been found to decline rapidly in activity and only regain activity 8 weeks after harvest in onion cvs. Copra and Golden Bear stored at 18°C (Yasin and Bufler, 2007). Pak *et al*. (1995) found that sucrose synthase decreased in activity after harvest and only increased again with the initiation of sprouting. This could explain the slight decrease in glucose and fructose concentration during curing as the reduction in sucrose synthase activity would cause a halt on the breakdown of sucrose into monosaccharides. Following curing the most significant sugar change was the increase in fructose concentration which could be due to the degradation of fructans into its component parts mostly made up of fructose. Benkeblia *et al*. (2002) noted a decrease in fructan concentration in onion cv. Rouge Amposta after 6 months of storage at 4°C, additionally Benkeblia *et al*. (2004) found the same polysaccharide decrease in onions cv. Jaune d'Espagne corresponding with a slight increase in tri- and tetra-saccharides.

The sugar profile suggests an initial reduction in enzymatic and metabolic activity which corresponds with a rapid reduction in respiration rate. The respiration rate of untreated onion bulbs dropped from 0.3 to 0.1 mmol CO_2 kg^{-1} hr^{-1} from day 0 to day 42. Benkeblia *et al*. (2004) found that after 24 hrs at 18°C the respiration rate of onion bulbs cv. Rouge Amposta was below 0.1 mmol CO_2 kg^{-1} hr^{-1}. This suggests that the dramatic reduction in respiration rate over the 42 days curing may have occurred within days of being transferred to the 28 °C curing conditions. Additionally, storage at 4°C maintained the respiration rate of the onion bulbs below 0.1 mmol CO_2 kg^{-1} hr^{-1} which storage at 10 °C and 20 °C did not. They also that found storage at 4 °C resulted in reduced sprouting and rotting which only became apparent after 16 weeks in store. It appears from the results reported here that during storage at 1 °C, the respiration rate remains low for both cultivars and all treatments, showing promising results for low levels of sprouting and rotting. The respiration rate of onions treated with ethylene before and after curing was significantly higher than the control which was not maintained beyond day 89. Ethylene has a similar effect on potato, also non-climacteric, resulting in a higher respiration rate (Daniels-Lake *et al*., 2005). Respiration causes an increase in reactive oxygen species (ROS) which are protected against by antioxidants. The decrease in respiration during curing may therefore be linked to the increase in antioxidant levels of the untreated onion bulbs as a reduction in ROS may cause the accumulation of antioxidants.

Total phenolics, total flavonoids and total antioxidant capacity all behaved similarly. Total phenolics and total flavonoids increased significantly in both cultivars during the 42 days of curing. Benkeblia (2000) found that during 20 °C storage, onion bulb phenolic concentration increased by an average of 0.1 mg per week between 0 and 10 weeks followed by a subsequent decrease, however, during storage at 4 °C, bulb phenolic content decreased at a rate of 0.52 mg per week between 0 and 10 weeks followed by a small increase of 0.06 mg per week. Each of these experiments were independent and storage temperatures not as hot as the 28 °C curing or as cold as the 1 °C storage used here, however it does support the hypothesis that hotter storage temperatures increase and colder temperatures decrease the total phenolic content of onions. It will be interesting to witness if there is a subsequent small increase of around 0.06 mg per week after 10 weeks in storage at 1 °C as seen by Benkeblia (2000).

Mogren *et al*. (2006) discovered that over a 10-14 day period of field curing (Temp. mean 16.7 °C, max 25.1 °C, min 10.6, RH mean 79, max 97, min 48), the quercetin glucosides levels almost doubled. Here we report that the total flavonoid levels for onions cv. Sherpa increased almost four-fold and cv. Wellington nearly three-fold.

The higher levels of flavonoids found here may be due to the longer period of curing, hotter curing temperature of 28 °C or differences between forced air curing and field curing.

After 105 days in cold store the total flavonoid and phenolic content and total antioxidant capacity of the onions was not reduced by any of the treatments when compared to the control which is important in terms of the consumer market and health benefits. It is equally important to the retailer and consumer that onion pungency is not affected by treatments and is measured in terms of pyruvate concentration. Pyruvate concentration has previously been described as irregular during storage (Benkeblia, 2000). The pungency of treated bulbs was not significantly different from the control bulbs following 42 days curing which is supported by Chope *et al*. (2007) who found onion bulbs cv. SS1 treated with 0.962 µl l^{-1} 1-MCP for 24 hrs showed no significant differences to the untreated bulbs at 4 °C.

Conclusions

Part 1: Gemma Chope, Leon Terry – CU (Objectives 1 and 4)

At this stage in the project we can say that the curing temperature has so far had no adverse effect on sprouting or disease incidence, or on the quality parameters measured, in onion bulbs cold-stored for 15 weeks. This means that substantial reductions in the use of propane gas for heating during curing could be made.

- Curing at a lower temperature did not resulted in an unacceptable skin finish or increased disease incidence.
- The proportion dry weight was not affected by treatment and so this indicates that curing at a lower temperature does not affect this parameter.
- Curing at a lower temperature did not affect pungency of onion bulbs.
- Onions grown at Elveden, and that were cured at 20ºC had greater glucose and fructose concentrations than those cured at 24 and 28ºC, but sucrose concentration was not affected by curing temperature.

Part 2: Gemma Chope – CU, Andrew Thompson, John Hammond – WHRI (Objective 2)

Genes selected for inclusion on the microarray chip such as those involved in ABA, ethylene and sugar metabolism tend to belong to multi-gene families. In general, the protein sequences that are encoded by these genes tend to form groups according to function, with subgroups according to species, suggesting that the sequences are fairly well conserved, and should also be expected to be conserved within onion.

Part 3: Kate Downes, Leon Terry – CU (Objective 3)

To date there is no indication that the ethylene and 1-MCP treatments have had any deleterious effects on the quality of the onion bulbs. We have been able to conclude that treatment with ethylene or 1-MCP prior to curing improves the efficiency of absorption and could prove more cost effective. As the experiment is not complete, toward the end of storage it will be possible to elucidate which treatment is most successful at reducing sprout incidence and disease incidence and gain insight into the pathways involved. What has been shown already is that the greatest period of change occurs immediately after harvest when the onions bulbs are subjected to high curing temperatures and entering a steady state of dormancy.

Technology transfer

- A poster and leaflet were produced for the 'Horticulture LINK 2007' event, on Wednesday 28th November at Lewis Media Centre, Millbank, London SW1.

Technology transfer has been limited as the project is still collecting data from the first storage season.

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Appendix 1

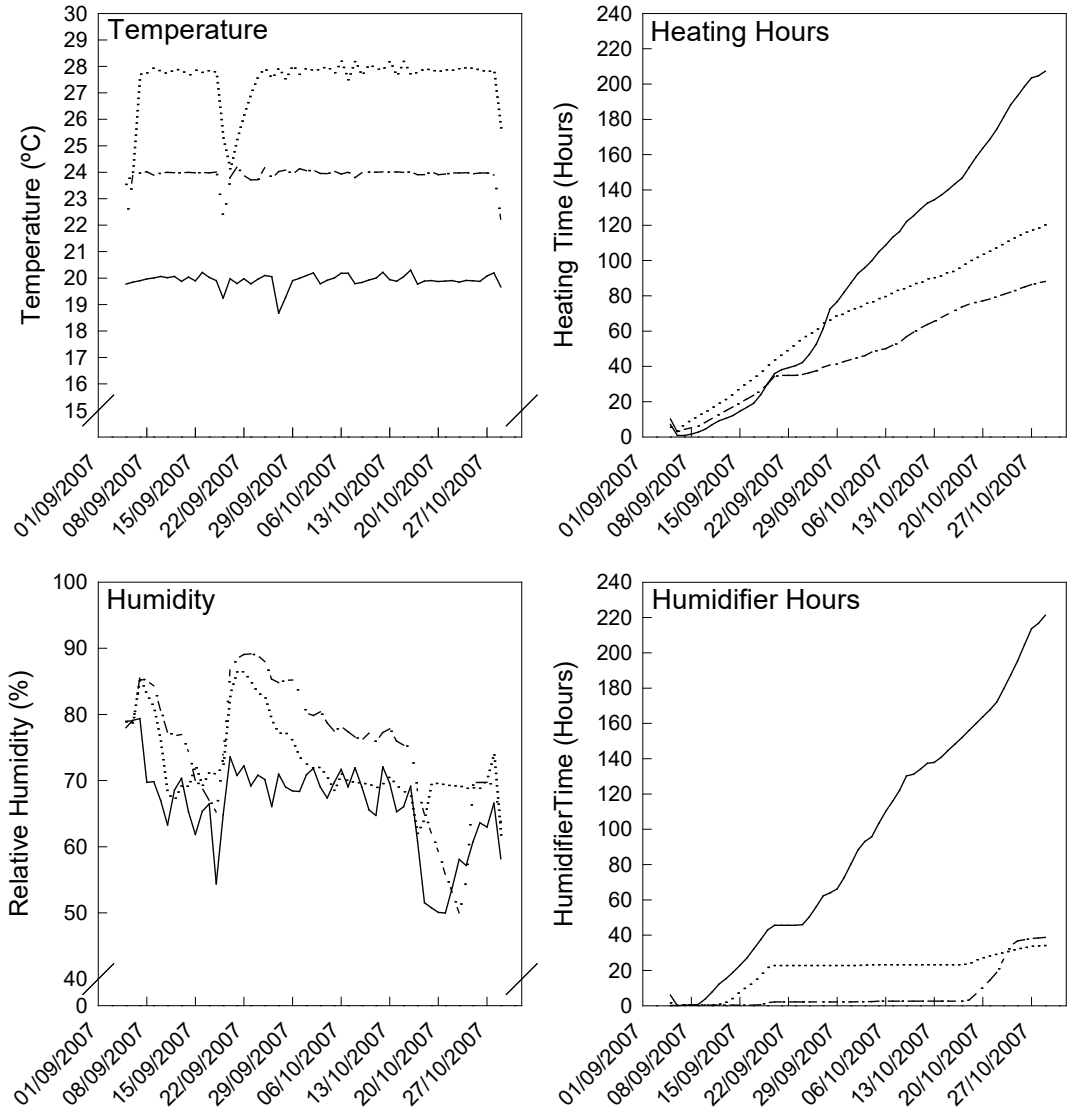

Figure A.1. The temperature, heating hours, humidity and humidifier hours for the stores set at 20 ºC (solid line), 24 ºC (dashed line) and 28 ºC (dotted line) used for curing at Sutton Bridge Experimental Unit.

Appendix 2

Elveden crop diary

V 3.83 / 0	Farmade MultiCrop For Elveden Farms Ltd Thetford Elveden Farms Ltd 2006/07	17/10/2007 Page 1

Field Traceability

Field	**Rake Btm Heath**	Type	**Main Field**	Working Area	**19.78Ha**
Reference	**189**	Variety	**S.Onions**		
Descriptor		Crop	**ONIONS**		

Date	Product	Harvest Interval	Ha	Rate/Ha	Units	GS	Water Vol	Active MAPP / Active Ingredient
15/02/2007	Glyphosate 360	0	19.54	1.500	Lt		200	12680 Glyphosate
13/03/2007	Propachlor	0	19.75	6.000	Lt		200	4873 Propachlor
Problem	Residual herbicide / volunteer cereals							
13/03/2007	Stomp 400 SC	0	19.75	0.800	Lt		200	13405 Pendimethalin
Problem	Residual herbicide / volunteer cereals							
13/03/2007	Defy	0	19.75	2.000	lt		200	12606 Prosulfocarb
Problem	Residual herbicide / volunteer cereals							
13/03/2007	PDQ	0	19.75	1.000	Lt		200	10532 Diquat+Paraquat
Problem	Residual herbicide / volunteer cereals							
14/04/2007	Stomp 400 SC	0	19.66	0.800	Lt		200	13405 Pendimethalin
Problem	Residual top up							
28/04/2007	Totril	14	19.66	0.350	Lt		250	10026 Ioxynil
Problem	Nettle control							
03/05/2007	Totril	14	19.77	0.350	Lt		240	10026 Ioxynil
Problem	Knotgrass / nettle control							
03/05/2007	Starane 2	77	19.77	0.200	lt		240	5496 Fluroxypyr
Problem	Knotgrass / nettle control							
08/05/2007	Falcon	28	19.66	0.700	Lt		200	10585 Propaquizafop
Problem	Foliar Feed / volunteer cereal control							
08/05/2007	MnSO4 liquid	0	19.66	2.000	Lt		200	Manganese sulphate
Problem	Foliar Feed / volunteer cereal control							
08/05/2007	MAG Sul pow	0	19.66	3.052	Kg		200	Magnesium sulphate
Problem	Foliar Feed / volunteer cereal control							
20/05/2007	Totril	14	19.66	0.500	Lt		240	10026 Ioxynil
Problem	Nettle control							
06/06/2007	Totril	14	19.66	0.600	Lt		240	10026 Ioxynil
Problem	BLW control							
06/06/2007	Fortrol	56	19.66	0.200	Lt		240	10666 Cyanazine
Problem	BLW control							
11/06/2007	Aramo	28	19.68	1.000	Lt		200	10280 Tepraloxydim
Problem	Foliar feed / AMG control							
11/06/2007	MnSO4 liquid	0	19.68	2.000	Lt		200	Manganese sulphate
Problem	Foliar feed / AMG control							
18/06/2007	Dithane 945	28	19.66	2.000	kg		200	12585 Mancozeb
Problem	Protectant fungicide							
18/06/2007	MnSO4 liquid	0	19.66	2.000	Lt		200	Manganese sulphate
Problem	Protectant fungicide							

Field Reference Descriptor	**Rake Btm Heath 189**		Type Variety Crop	**Main Field S.Onions ONIONS**	Working Area	**19.78Ha**

						Active
18/06/2007	Bond	0	19.66	0.200 Lt	200	
Problem	Protectant fungicide					
22/06/2007	Stomp 400 SC	0	19.58	0.500 Lt	200	13405 Pendimethalin
Problem	Residual herbicide top up					
22/06/2007	Atlas CIPC 40	14	19.58	0.500 Lt	200	7710 Chlorpropham
Problem	Residual herbicide top up					
30/06/2007	Amistar	14	19.66	0.500 Lt	200	10443 Azoxystrobin
30/06/2007	Dithane 945	28	19.66	1.999 kg	200	12585 Mancozeb
30/06/2007	MnSO4 liquid	0	19.66	1.999 Lt	200	Manganese sulphate
30/06/2007	Bond	0	19.66	0.200 Lt	200	
04/07/2007	Linuron 50 SC	56	19.66	0.350 Lt	500	6967 Linuron
Problem	Nettle control					
11/07/2007	Amistar	14	19.66	0.500 Lt	200	10443 Azoxystrobin
11/07/2007	Invader	7	19.66	2.000 Kg	200	10390 Dimethomorph+Mancozeb
11/07/2007	Bond	0	19.66	0.200 Lt	200	
18/07/2007	Aramo	28	19.66	0.500 Lt	200	10280 Tepraloxydim
Problem	Protectant fungicide / AMG control					
18/07/2007	Folio Gold	14	19.66	2.000 lt	200	10704 Metalaxyl-M+Chlorothalonil
Problem	Protectant fungicide / AMG control					
18/07/2007	Amistar	14	19.66	0.500 Lt	200	10443 Azoxystrobin
Problem	Protectant fungicide / AMG control					
18/07/2007	Dithane 945	28	19.66	2.000 kg	200	12585 Mancozeb
Problem	Protectant fungicide / AMG control					
18/07/2007	MnSO4 liquid	0	19.66	2.000 Lt	200	Manganese sulphate
Problem	Protectant fungicide / AMG control					
28/07/2007	Folio Gold	14	19.66	2.000 lt	200	10704 Metalaxyl-M+Chlorothalonil
Problem	Protectant fungicide					
28/07/2007	Amistar	14	19.66	0.500 Lt	200	10443 Azoxystrobin
Problem	Protectant fungicide					
28/07/2007	Invader	7	19.66	2.000 Kg	200	10390 Dimethomorph+Mancozeb
Problem	Protectant fungicide					
02/08/2007	Folio Gold	14	19.78	2.000 lt	200	10704 Metalaxyl-M+Chlorothalonil
02/08/2007	Dithane 945	28	19.78	2.000 kg	200	12585 Mancozeb
09/08/2007	Invader	7	19.66	2.000 Kg	200	10390 Dimethomorph+Mancozeb
16/08/2007	Invader	7	19.66	2.000 Kg	200	10390 Dimethomorph+Mancozeb

Field	**Rake Btm Heath**	Type	**Main Field**	Working Area	**19.78Ha**
Reference	**189**	Variety	**S.Onions**		
Descriptor		Crop	**ONIONS**		

Active

Safe Harvest Date - 30/08/2007

Actual Harvest - 30-31/08/07

Date	Value	Comment

ALLIUM AND BRASSICA CENTRE

Application Analysis Report

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Indlay & Co., Lakes Farm, Cardington, BEDFORD

Field: GR126 * * *
Crop: Spr Bulb Onions, Drilled
Field Ref:

Field Area: 21.50 ha
Drilled Area: 21.50 ha (26/03/2007)

Harvested: 08/09/2007
Earliest Harvest Date: 02/09/2007

Applied	No	Operator	GS	Area	Product	Active	Reason	Rate	Water Vol	S.O.L.A. No	HI Days
/03/2007	141	A Powell		21.50	RAMROD FLOWABLE	Propachlor	Residual herbicide	9.00 lts/ha	200 Lts/ha		
/03/2007	141	A Powell		21.50	STOMP 400 SC	Pendimethalin	Residual herbicide top up	1.00 lts/ha	200 Lts/ha	2313/04	
/05/2007	113	A Powell		21.50	STOMP 400 SC	Pendimethalin	Residual herbicide top up	0.80 lts/ha	200 Lts/ha	2313/04	
/05/2007	113	A Powell		21.50	PYRAMIN DF	Chloridazon	Residual herbicide top up	0.50 Kgs/ha	200 Lts/ha	0732/97	
/05/2007	117	A Powell		21.50	STOMP 400 SC	Pendimethalin	Residual herbicide top up	0.80 lts/ha	200 Lts/ha	2313/04	
/05/2007	117	A Powell		21.50	PYRAMIN DF	Chloridazon	Residual herbicide top up	0.40 Kgs/ha	200 Lts/ha	0732/97	
/05/2007	117	A Powell		21.50	TOTRIL	Ioxynil	Broad leaved weed control	0.15 lts/ha	200 Lts/ha		14
/06/2007	122	A Powell		21.50	STARANE 2	Fluroxypyr	Cleavers	0.40 lts/ha	200 Lts/ha	0986/04	77
/06/2007	122	A Powell		21.50	BASAGRAN SG	Bentazone	Mayweed	0.40 kgs/ha	200 Lts/ha	1631/06	21
/06/2007	123	A Powell		21.50	ARAMO	Tepraloxydim	Annual Meadow Grass Control	1.50 lts/ha	200 Lts/ha		28
/06/2007	123	A Powell		21.50	MANGANESE SULPHATE		Manganese deficiency detectable	5.00 kgs/ha	200 Lts/ha		
/06/2007	130	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Lts/ha	2334/04	7
/06/2007	130	A Powell		21.50	AMISTAR	Azoxystrobin	Protectant fungicide	1.00 lts/ha	200 Lts/ha		14
/06/2007	130	A Powell		21.50	MANGANESE SULPHATE		Manganese deficiency detectable	3.00 kgs/ha	200 Lts/ha		
/06/2007	130	A Powell		21.50	CUPROKYLT	Copper Oxychloride	Bacterial Control	2.00 kgs/ha	200 Lts/ha	1127/99	14
/03/2007	137	A Powell		21.50	FOLIO GOLD	Chlorothalonil Metalaxyl-m	Downy Mildew	2.00 lts/ha	200 Lts/ha		14
/03/2007	137	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Lts/ha	2334/04	7
/03/2007	137	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Lts/ha	0415/02	14
/03/2007	137	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Lts/ha		
/03/2007	138	A Powell		21.50	AMISTAR	Azoxystrobin	Protectant fungicide	1.00 lts/ha	200 Lts/ha		14
/03/2007	138	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Lts/ha	2334/04	7
/03/2007	138	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Lts/ha	0415/02	14
/03/2007	138	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Lts/ha		
/03/2007	142	A Powell		21.50	FOLIO GOLD	Chlorothalonil Metalaxyl-m	Downy Mildew	2.00 lts/ha	200 Lts/ha		14

* * Shows Fields With Unconfirmed Plans

Print Date 17/12/2007

ALLIUM AND BRASSICA CENTRE

Application Analysis Report

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Hadlay & Co., Lakes Farm, Carlington, BEDFORD

Field: OR126 * * *
Crop: Spr Bulb Onions, Drilled
Field Ref:

Field Area: 21.50 ha
Drilled Area: 21.50 ha (26/03/2007)

Harvested: 08/09/2007
Earliest Harvest Date: 02/09/2007

Applied	No	Operator	GS	Area	Product	Active	Reason	Rate	Water Vol	S.O.L.A. No	HI Days
02/2007	142	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Ltr/ha	2334/04	7
02/2007	142	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Ltr/ha	0415/02	14
02/2007	142	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Ltr/ha		
02/2007	139	A Powell		21.50	BRAVO 500	Chlorothalonil	Protectant fungicide	2.00 lts/ha	200 Ltr/ha		14
02/2007	139	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Ltr/ha	2334/04	7
02/2007	139	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Ltr/ha	0415/02	14
02/2007	139	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Ltr/ha		
03/2007	143	A Powell		21.50	FOLIO GOLD	Chlorothalonil Metalaxyl-m	Downy Mildew	2.00 lts/ha	200 Ltr/ha		14
03/2007	143	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Ltr/ha	2334/04	7
03/2007	143	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Ltr/ha	0415/02	14
03/2007	143	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Ltr/ha		
03/2007	144	A Powell		21.50	BRAVO 500	Chlorothalonil	Protectant fungicide	2.00 lts/ha	200 Ltr/ha		14
03/2007	144	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Ltr/ha	2334/04	7
03/2007	144	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Ltr/ha	0415/02	14
03/2007	144	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Ltr/ha		
03/2007	145	A Powell		21.50	BRAVO 500	Chlorothalonil	Protectant fungicide	2.00 lts/ha	200 Ltr/ha		14
03/2007	145	A Powell		21.50	INVADER	Dimethomorph Mancozeb	Downy Mildew	2.00 Kgs/ha	200 Ltr/ha	2334/04	7
03/2007	145	A Powell		21.50	HEADLAND INORGANIC LIQUID COPPER	Copper Oxychloride	Bacterial Control	2.00 lts/ha	200 Ltr/ha	0415/02	14
03/2007	145	A Powell		21.50	MANGANESE LIQUID 15%	Manganese	Manganese deficiency detectable	3.00 lts/ha	200 Ltr/ha		

Of Water Courses In Field Dry Ditches: 0 Less Than 3m: 0 Between 3m and 6m: 0 More than 6m: 0

* * Shows Fields With Unconfirmed Plans

Print Date 17/12/2007